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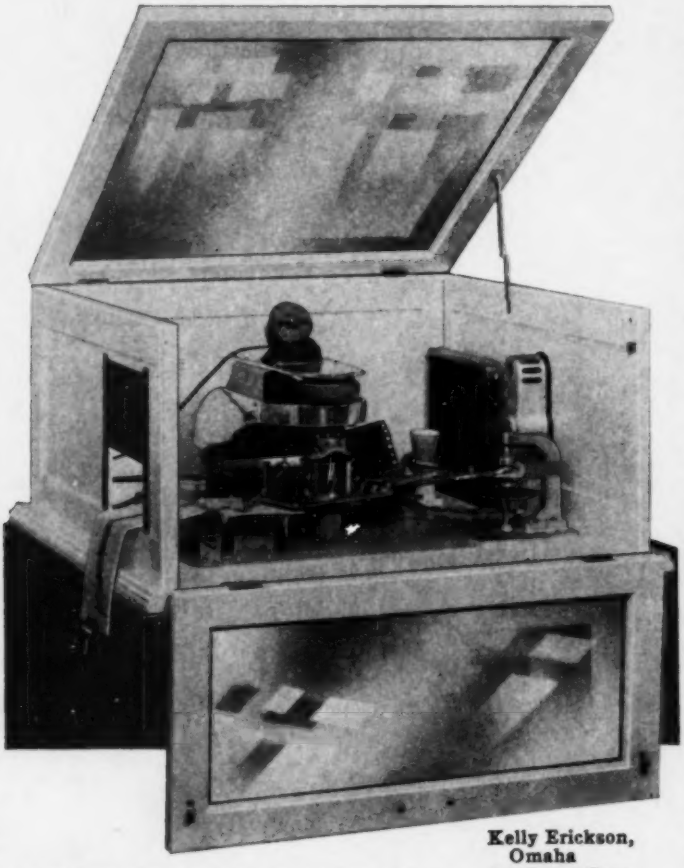

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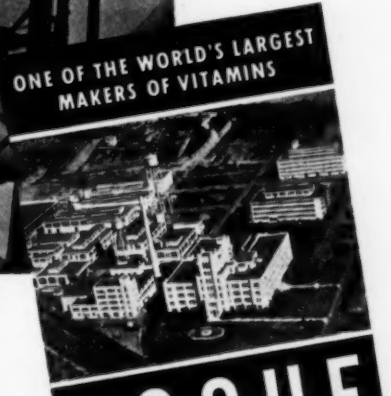

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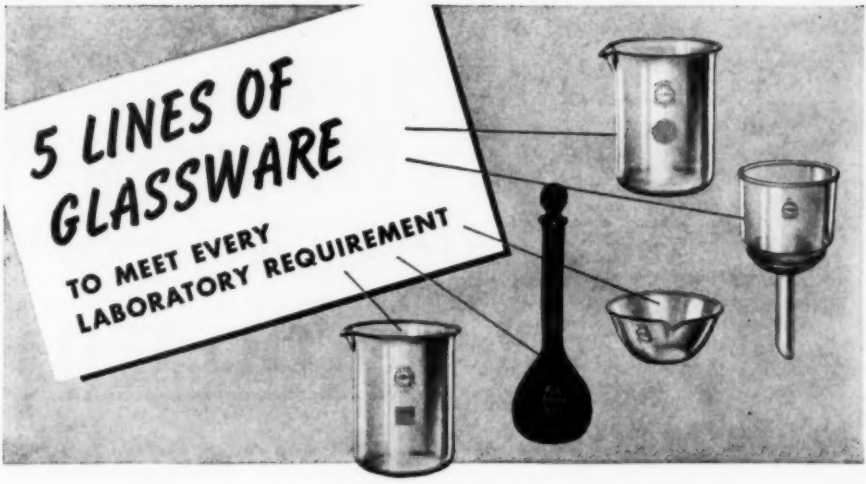

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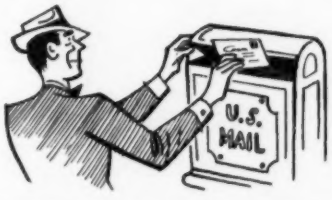

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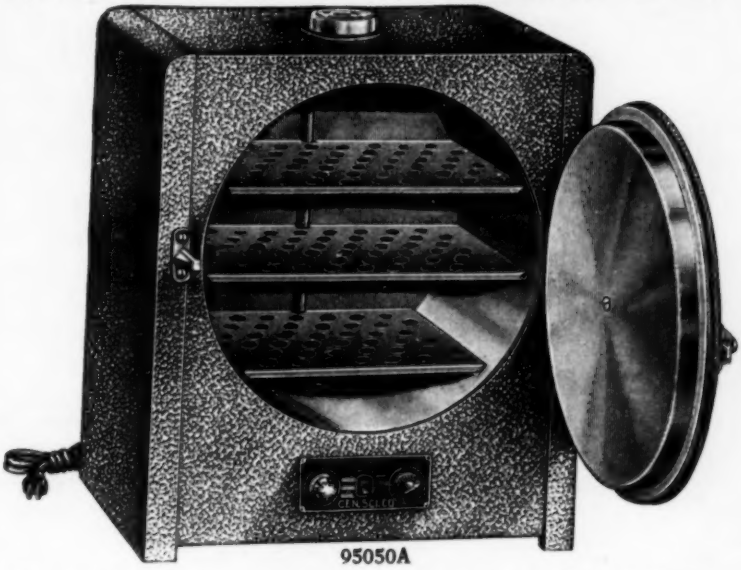

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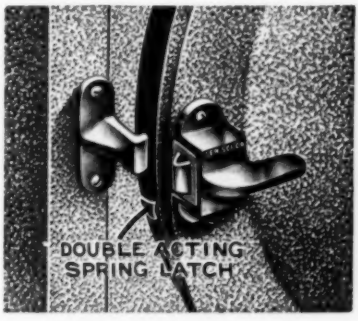

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CEREAL CHEMISTRY

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BIOCHEMISTRY OF THE DAMAGE TO GRAIN BY THE WHEAT-BUG

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(Institute of Biochemistry, Academy of Sciences, Moscow, U.S.S.R.)

(Received for publication April 6, 1943)

One of the most serious types of wheat damage in Europe, Asia, and North Africa is that caused by insects of such species as Eurygaster, Aelia, and Dolycoris, first described by Berliner (1931). These insects, generally called wheat-bugs, attack the kernel during the ripening period and lower its viability and baking quality. The data obtained by Kretovich, Bundel, and Pschenova (1943), reproduced in Table I, illustrate the reduction in viability.

TABLE I

VIABILITY OF WHEAT INFECTED BY THE WHEAT-BUG

Sample	Infected kernels	Viability		
		Entire sample	Normal kernels	Damaged kernels
	%	%	%	%
1	3.8	94.8	95.0	89.1
2	8.6	92.1	96.0	50.9
3	13.1	90.7	98.0	41.8
4	14.3	85.8	95.0	30.9
5	19.2	79.1	90.0	32.7
6	24.3	67.9	82.0	24.0

The deterioration in baking quality is manifested by the fact that the flour from highly damaged grain either does not form gluten or the gluten can hardly be washed out; it sticks to the fingers, has no elasticity, and upon standing, disintegrates into a sticky mass. Consequently, dough prepared from damaged flour also disintegrates, is hard to mold, and produces flat loaves of poor crumb texture.

Investigations have shown that wheat-bugs puncture the ripening kernel and suck out a portion of the contents. Consequently the

kernel, though ripening and reaching full maturity, is of inferior viability and baking quality.

After harvest, a wheat kernel which has been punctured by the wheat-bug is shrivelled, has lower absolute weight, and shows a pale area with or without a small dark spot on its surface. The pale area is usually slightly shrivelled and is limited to the damaged region. The endosperm in the damaged region is very soft and can be easily crumbled out, whereas in the undamaged part of the kernel it is normally hard. If the kernel has been pierced many times by the wheat-bug it contains almost no endosperm.

Upon examining a cross section through the pierced area of a damaged kernel under the microscope, one may see that a considerable part of the protein in that area has been dissolved (Fleckinger, 1936). It is natural to suppose that the bug, after having punctured the kernel, injects secretions which cause the dissolution of the contents of the endosperm cells. Actually, Hibraoui (1930) and Malenotti (1933) have observed that wheat-bugs inject a liquid when piercing the kernel.

The experiments reported here were designed to determine the biochemical properties of grain damaged by wheat-bugs.

Experimental

In studying the effect of the wheat-bug on the protein complex of the kernel the first consideration is to examine the total nitrogen in the damaged kernel. The analysis of normal and infected kernels, selected by hand, showed that the total nitrogen content is decreased as a result of the damage caused by the wheat-bug (Table II).

TABLE II

Total Nitrogen Content of Normal and Infected Kernels

Sample	Nitrogen [1]	
	Normal	Infected
	%	%
1	2.03	1.88
2	2.28	2.02
3	2.43	2.14
4	2.24	2.07
5	2.26	2.10
6	2.17	1.95

[1] Dry matter basis.

These results indicate that wheat-bugs, in piercing the kernel, extract part of the nitrogenous substances.

More detailed analyses of normal and infected kernels are given in Table III.

These data show that the titratable acidity of flour from damaged grain is higher than that for normal grain. The amylase activity is also higher in the flour from infected grain, indicating that the wheat-bug introduces amylase into the kernel.

TABLE III

BIOCHEMICAL ANALYSES OF NORMAL AND INFECTED KERNELS

Characteristics of kernels	Nitrogen				Nitrogen of gliadin as percent of total gluten nitrogen	pH of flour suspension	Titratable acidity of extract		Activity of	
	Total	Water-soluble, as percent of total	Gliadin nitrogen as percent of total	In gluten			Water	Alcohol	Catalase[1]	Amylase[2]
	%	%	%	%	%					
Normal kernels	2.70	14.54	47.2	15.3	51.6	6.6	2.3	3.0	38.9	114.4
Damaged kernels (with pale areas and dark spots)	2.59	28.0	54.2	14.6	71.8	6.5	2.7	4.2	37.8	163.6
Damaged kernels (without dark spots)	2.51	29.8	55.1	—	—	—	—	—	—	—

[1] Method of Bach and Oparin (1923).
[2] Method of Rumsey (1922).

The nitrogen fractionation studies show that infection by the wheat-bug considerably increases the solubility of the proteins in both water and 60% alcohol. It is thus clear that the nitrogenous substances of the damaged kernels in particular underwent profound changes; the gluten had about 75% of gliadin nitrogen instead of the usual 50%.

Since the high water-soluble nitrogen values for the infected kernels suggested extensive proteolysis, it seemed necessary to examine the relative changes in nitrogen solubility which occurred when normal and infected wheats were subjected to autolysis. As a measure of proteolytic activity, the total water-soluble nitrogen and the nitrogen not precipitated by 2% trichloracetic acid solution were determined before and after autolysis. The results given in Table IV demonstrate the considerable increase (during autolysis) of nitrogen not precipitated by trichloracetic acid. This is characteristic of peptones, peptides, and amino acids formed as a result of extensive enzymatic splitting of proteins in the damaged grain. In line with these data, Geoffroy (1936) reported a greater increase in amino nitrogen during the autolysis of damaged grain as compared with normal grain.

To determine more definitely the changes which the protein substances undergo when subjected to the effect of wheat-bugs, pure

gliadin preparations were made from normal and infected kernels by Kretovich's (1937) acetone method. The very process of obtaining the preparations confirmed the greater solubility of the proteins from infected kernels in comparison with the controls: a yield of only 2.6 g

TABLE IV

CHANGE IN SOLUBLE NITROGEN FRACTIONS OF NORMAL AND DAMAGED KERNELS DURING AUTOLYSIS

Sample No.	Kernels	Nitrogen in percent of total nitrogen			
		Before autolysis		After autolysis	
		Water-soluble	Not precipitated by CCl_3COOH	Water-soluble	Not precipitated by CCl_3COOH
		%	%	%	%
1	Normal	12.5	7.6	22.7	16.1
	Damaged	22.0	16.3	52.7	43.8
2	Normal	10.0	4.7	21.2	13.4
	Damaged	19.9	14.0	56.9	42.3

of gliadin was secured from 250 g of infected kernels, whereas from 250 g of normal kernels, under the same conditions, 6.7 g was obtained. The sharp reduction in gliadin yield from infected kernels may be explained by its dispersion in water during washing.

Investigation of the physical and chemical properties of the gliadins showed (Table V) that the protein of the infected kernels under-

TABLE V

PROPERTIES OF GLIADIN FROM NORMAL AND INFECTED KERNELS

Source of gliadin	Nitrogen	Sulfur	Tryptophane (Furth Diesche)	Viscosity[1]	$[\alpha]_D^{20}$ in 60% ethanol
	%	%	%		
Normal kernels	17.6	0.97	0.91	3.008	−91.8
Infected kernels	17.1	1.37	0.87	2.737	−72.5

[1] Viscosity values are given as the ratio of the viscosity of 1% gliadin solution in 60% alcohol to the viscosity of water.

goes deep intramolecular changes which result in a considerable lowering of the viscosity and specific rotation of gliadin solutions as well as in a marked increase in the sulfur content of the gliadin. The decrease in viscosity and the change in optical activity of protein solutions during autolysis are recognized phenomena which may serve as quantitative measures of proteolysis. It is possible, of course, that the pronounced differences in the optical properties of the gliadin prepared

from normal and infected kernels may be caused by the presence of glutenin cleavage products in the gliadin prepared from the damaged kernels.

The high sulfur content of the gliadin from the infected kernels is of particular interest. Evidently the proteolysis caused by the secretions of the wheat-bug does not involve the liberation of sulfur-containing amino acids, such as cystine and methionine, from the protein molecule. A similar observation was made by Chittenden and Smith (1890) who found that the colloidal products of the hydrolysis of wheat glutenin by pepsin contained much more sulfur than the original protein.

The question arises whether the secretions of the bug are concentrated in the region of the puncture or whether they extend throughout

TABLE VI

NITROGEN FRACTIONS OF NORMAL AND DAMAGED PARTS OF INFECTED WHEAT KERNELS

Sample No.	Part of kernel	Total nitrogen	Alcohol-soluble nitrogen as percent of total nitrogen	Water-soluble nitrogen as percent of total nitrogen	Nitrogen not precipitated by CCl_3COOH as percent of total nitrogen
		%	%	%	%
1	Normal	2.56	40.1	12.9	9.1
	Infected	1.28	47.2	47.4	41.0
2	Normal	2.76	39.3	17.2	14.8
	Infected	1.14	44.6	57.3	54.2
3	Normal	2.84	43.4	19.1	15.6
	Infected	1.39	53.5	59.8	54.2

the whole kernel. In order to answer this question infected kernels were dissected into two parts: one entirely normal to all appearance, and the other showing evident signs of infection. Both fractions were then ground separately and the gluten washed out. It was not possible to wash out even traces of gluten from the infected parts of the kernel, whereas, from the undamaged parts, the gluten washed out quite normally, although it was somewhat weak. These experiments show that the secretions injected by the bug do not disperse throughout the kernel but localize in the area of infection. Accordingly, one would suspect that the deep-seated changes in the nitrogenous constituents of the kernel would also be limited to this area.

To verify this suggestion undamaged parts of the kernel as well as those immediately adjacent to the point of attack were excised. These normal and damaged fractions were then ground and subjected to analyses, the results of which are summarized in Table VI.

It is readily apparent that the infected areas of the kernels are lower in total nitrogen than the normal portions and, also, that the nitrogenous constituents are less complex. On the average, approximately one-half of the total nitrogen of the infected portions of the kernels is water-soluble and not precipitable with 2% trichloracetic acid solution.

Evidence of extensive proteolysis in infected kernels has led to attempts to establish a simple method for diagnosing the amount of damage by the bug; this method is based on color reactions for amino acids, the presence of which might be expected in the infected area.

The experiments of Tokareva (1940) showed that on heating a water extract of infected kernels with Millon's reagent, a red coloration develops in the solution and in the protein coagulum formed during heating; in the instance of noninfected kernels only the coagulum is colored. This difference in behavior may be explained as being due to the enzymatic liberation of tyrosine either in the free form or as a peptide. The ready liberation of tyrosine from protein by proteolytic

TABLE VII

TYROSINE CONTENT OF NORMAL AND INFECTED KERNELS

Sample No.	Tyrosine[1] after precipitation by 2% CCl_3COOH		Tyrosine[2] after precipitation by $Hg(NO_3)_2$		Infected area of kernel
	Normal kernels	Infected kernels	Normal kernels	Infected kernels	
	mg/g	*mg/g*	*mg/g*	*mg/g*	*mg/g*
1	0.34	0.51	0.00	0.04	—
2	0.50	1.13	0.00	0.04	—
3	0.60	1.80	0.00	0.06	0.09
4	0.58	1.73	0.00	0.05	0.08

[1] Method of Zuverkalow (1927).
[2] Method of Thomas (1921).

enzymes was shown by Northrop and his associates (1939); it is, therefore, quite natural that the extensive proteolysis taking place in the kernel infected by the wheat-bug is accompanied by the accumulation of tyrosine in the free and peptide form. This fact was confirmed by Goldstein, Gorbachevskaya, and Sklovskaya (1940).

More detailed data characterizing the content of tyrosine in the normal and infected wheat kernel were obtained by Kretovich, Bundel, and Pschenova (1943) and are given in Table VII. The amount of tyrosine not precipitated by protein precipitants is much higher in infected than in normal kernels. After precipitating extracts from the kernels by means of a mercury salt which is a very strong precipitant, a much smaller content of tyrosine was found than after precipitation with CCl_3COOH. This proves that the greater part of the tyrosine

found in the infected kernel belongs to the colloidal peptones and other cleavage products of relatively high molecular weight which are formed during the enzymatic splitting of protein.

What then are the essential reasons for the drastic degradation of gluten in the infected grain? Berliner (1931), having discovered the gluten degradation caused by the wheat-bug, was the first to suggest that this phenomenon is due to the presence of powerful proteolytic enzymes in the infected kernels. However, he was unable to prove this by adding water extracts of infected kernels to normal flour, since the gluten-forming property of the flour was not destroyed. Subsequently, the enzymatic nature of the injuries was suggested by Kosmin and Markin (1934) and by Blagoveshchenskii and Sosiedov (1934).

In checking the activity of water extracts, it was found that they acted weakly on the gluten of normal flour, but more active water extracts were obtained by a prolonged autolysis of ground infected kernels in small quantities of water. It may be presumed, therefore, that there exists a specific relation between the colloids of the infected kernel and its proteolytic enzymes, and that salt solutions [employed by Willstätter and Rohdewald (1932) for the extraction of leucocyte-desmoproteases which are closely associated with cellular structures] must be used to effect more rapid and complete extraction of the proteases.

After numerous experiments it appeared that 0.5% sodium carbonate solution was the most suitable solvent; extremely active preparations were obtained by extracting ground infected kernels for 30 to 60 min. The effect of these extracts on gluten may be illustrated by citing the results of a typical experiment.

Normal and infected kernels, selected from one sample, were ground and then extracted in the ratio of 1 : 5 with 0.5% Na_2CO_3 solution for 15 hours at 30°C in the presence of thymol. Doughs were then made with the filtered extracts and a strong flour. After a 30-min rest period in a thermostat, the glutens were washed out and their extensibilities determined by the method of Kosmin (1934) as modified by Kranz (1935). The gluten from the dough made with an extract from the normal kernels had an extensibility of 0.47 cm/min; the gluten from the dough made with an extract from the infected kernels, 31.3 cm/min; and the gluten from the dough made with an extract from infected kernels heated for 15 min at 100°C, 0.10 cm/min. When the doughs were allowed to rest for 3 hours at 30°C, that made with the extract from the infected kernels did not give any gluten. Thus it is apparent that the kernels infected by the wheat-bug contain extremely active proteolytic enzymes which are easily destroyed by heating; in the present experiment, the proteolytic activity of the

extract from infected kernels was 66 times higher than that from normal kernels.

In order to compare the proteolytic activity of water and sodium carbonate extracts of ground wheats containing various types of damage, the extracts were used in making doughs with a flour milled from sound wheat which yielded a strong, elastic gluten. The doughs were allowed to stand and their extensibilities determined by the Kosmin-Kranz method (Kosmin, 1934; Kranz, 1935). The results of these tests, given in Table VIII, show that wheat infected by the wheat-bug

TABLE VIII

PROTEOLYTIC ACTIVITY OF DIFFERENT TYPES OF WHEAT

Type of wheat	Extensibility of gluten from dough made with the extract	
	Water	Na_2CO_3
	cm/min	*cm/min*
Control[1]	0.01	0.02
Normal	0.04	0.03
	0.03	0.02
	0.03	0.03
	0.02	0.07
100% infected by other insects	0.03	0.01
	0.02	0.06
Germinated	0.05	0.03
	0.01	0.01
	0.04	0.02
	0.07	0.03
	0.09	0.03
Frosted	0.12	0.03
	0.02	0.05
Drouth-damaged	0.05	0.03
	0.04	0.02
	0.04	0.01
	0.03	0.02
	0.02	0.02
100% infected by wheat-bugs	0.76	1.17
	1.25	2.00
	0.50	2.75
	4.20	17.00
	19.20	30.00

[1] Dough made with pure water or 0.5% Na_2CO_3 solution.

can easily be distinguished from any other type of damage by the high proteolytic activity of both water and sodium carbonate extracts; moreover, in the instance of wheat-bug damaged grain, the water extracts are lower in proteolytic activity than the sodium carbonate extracts. These observations establish beyond doubt that gluten degradation is caused by the high proteolytic activity of wheat in-

fected by the wheat-bug; also, that there are insignificant quantities of soluble proteases in wheat containing other types of damaged kernels.

Having demonstrated the high proteolytic activity of sodium carbonate extracts of infected grain, an attempt was made to accelerate the test. The extract was prepared by adding the sodium carbonate solution, previously heated to 40°C, to the ground wheat and allowing the suspension to stand in a thermostat at 37°C for 30 min. Doughs made with the filtered extracts were left in a thermostat maintained at 30°C for 30 min; the gluten was then washed out with water at 20°C and tested according to the Kosmin-Kranz method. The results of the accelerated technique given in Table IX show a still more

TABLE IX

PROTEOLYTIC ACTIVITY OF DIFFERENT WHEATS AS MEASURED BY THE ACCELERATED TECHNIQUE

Type of grain used for obtaining the extract	Extensibility of the gluten
	cm/min
Control [1]	0.05
Normal	0.03
	0.02
	0.04
	0.03
	0.09
Germinated	0.02
	0.12
Frosted	0.04
Drouth-damaged	0.05
100% infected by the wheat-bug	195.
	116.

[1] Dough made with 0.5% Na_2CO_3 solution.

striking difference than those recorded in Table VIII. The method may be recommended as a more convenient means for diagnosing infection by the wheat-bug and estimating the proteolytic activity of the grain than procedures based upon the determination of protein cleavage products. At the same time, it reflects the first stages of proteolysis which are of most interest to the baker and which are not measured by many other methods for the quantitative determination of proteolytic activity. The precision of this method could be increased by replacing the Kosmin-Kranz extensibility test with a determination of the physical properties of the dough in an apparatus such as the alveograph or farinograph.

Our experiments with sodium carbonate extracts refute the assumption that the high proteolytic activity of the flour from infected grain

is due to the increased content of sulfhydryl compounds. To secure definite proof that sulfhydryl compounds play no part in the processes of gluten degradation in wheat-bug infected kernels, the quantity of such compounds was determined in the flour from infected and normal kernels by a modification of the method of Woodward and Fry (1932). The results showed that the high activity of proteinases in infected kernels could not be explained by an increased content of sulfhydryl compounds.

Since it has been determined that the degradation of gluten in the infected kernel is due to the presence of extremely active proteolytic enzymes, it is appropriate to study the conditions of their activity, especially in relation to pH and temperature. The methods generally used for determining proteolysis, based on the determination of free amino groups, could not be effective in the present case as they do not

TABLE X

Effect of pH on Proteinase Activity of Infected Wheat

pH	Nitrogen dissolved by enzyme action [1]	
	Test 1	Test 2
	mg	*mg*
3.2	0.46	1.44
4.2	2.94	—
5.2	3.46	4.33
6.2	4.55	—
7.2	4.89	4.75
8.0	4.01	—

[1] Per 5-ml solution.

record the action of enzymes during the first stages of proteolysis. The following method was therefore employed: 9 ml of glycerol extract (40% glycerol) from ground infected kernels was added to 0.5 g of finely ground vacuum-dried gluten; the pH of the mixture was brought to the desired value with 10 ml of buffer solution prepared according to McIlvaine (1921). Thymol was used as an antiseptic. Simultaneously, a control experiment was carried out under the same conditions, using 9 ml of 40% glycerol. The experimental and control tubes were placed in a thermostat at 36°C for 16 hours. The total nitrogen in 5 ml of the filtered solution was then determined, and from this the total nitrogen of the corresponding control and of the enzyme extract was deducted. The resulting value served as a measure of proteinase activity. Gluten, rather than edestin, gelatin, or other proteins, was used as a protein substrate since the researches of Northrop (1939) suggest that the activity of the enzyme on various proteins would be different. The results of two typical experiments, given in Table X,

show that a neutral or slightly alkaline reaction is most favorable for the action of the gluten-dissolving enzymes of infected wheat. More striking results can be obtained if the measurement of proteolytic activity is based on the effect of the solutions on gluten extensibility; very evident by this technique are the greater activity in an alkaline medium and the considerable decrease in activity upon acidification of the dough.

Experiments to determine the effect of temperature showed, as anticipated, that the proteinase activity decreased with the lowering of the temperature.

To obtain dry preparations of the proteolytic enzymes and to investigate their properties, sodium carbonate extracts of normal and infected kernels, obtained according to the method described above, were dialyzed against distilled water until all dialyzable substances were removed. The liquid in the collodion sack was saturated with a tenfold volume of ethanol and then acidified with several drops of 10% acetic acid solution until an abundant precipitate was obtained. The precipitate was washed several times with strong alcohol, then with anhydrous ether, and dried in a vacuum. The preparations were light-grey powders; the yield from normal kernels was 1.48% and from infected kernels, 1.88%.

When preparations from infected kernels amounting to 0.1% were added to flour having good gluten, the extensibility of gluten washed out of the dough after a 30-min rest period at 30°C increased from 2.25 cm/min to 24.0 cm/min. When 1.0% of this preparation was added to the same flour, it was impossible to wash gluten from the

TABLE XI

EFFECT OF DRIED ENZYME PREPARATIONS ON GLUTEN OF STRONG AND WEAK FLOURS

	Gluten extensibility	
	Strong flour	Weak flour
	cm/min	*cm/min*
Control	0.07	0.62
Control + 0.1% of preparation from normal grain	0.12	0.94
Control + 0.1% of preparation from infected grain	4.26	Gluten did not wash out

dough at the end of the rest period; a sticky incoherent mass, characteristic of doughs made from flours prepared from infected wheat, was obtained.

It was of special interest to investigate the effect of these dried enzyme preparations on the physical properties of gluten of strong and

weak wheat flours milled from sound wheat. Doughs containing 0.1% of the dry enzyme preparations from normal and infected wheat, respectively, were prepared with the two flours; after allowing them to stand at 18° to 20°C for 3 hours, the gluten was washed out and tested according to the Kosmin-Kranz method. The results, recorded in Table XI, are very significant and demonstrate the fallacy of estimating the quality of infected grain by recording the percentage of infected kernels in a given blend, as well as the impossibility of establishing any safe maximal infection applicable to every type of wheat.

To study directly the influence of infected grain on the deterioration of glutens of varying quality, 0.5, 1.3, and 6% of infected kernels were added to four wheats, designated as Nos. 1, 2, 3, and 4 in order of decreasing strength. The wheats were then ground and doughs were prepared. After the doughs had rested at 30°C for 30 min, the glutens were washed out and their extensibilities determined by the Kosmin-Kranz method under strictly comparable conditions.

The results, given in Table XII, clearly show that the initial quality of the wheat is the most important factor in relation to the damage

TABLE XII

INFLUENCE ON GLUTEN QUALITY OF THE ADDITION OF INFECTED KERNELS TO WHEATS OF VARYING STRENGTH

Sample No.	Wheat type	Gluten extensibility				
		Percentage of infected kernels				
		0%	0.5%	1%	3%	6%
		cm/min	*cm/min*	*cm/min*	*cm/min*	*cm/min*
1	Strong	0.01	0.01	0.02	0.30	2.3
2	Intermediate	0.03	0.03	0.25	0.80	2.5
3	Fairly weak	0.06	0.90	0.90	13.40	41.1
4	Weak	0.30	2.10	2.70	20.70	50.0

caused by the wheat-bug; even after the addition of 6% of infected grain, the glutens from the two strongest wheats (Nos. 1 and 2) were normal, whereas those from the weakest wheats (Nos. 3 and 4) were originally poor and could only be washed out with great difficulty. From the standpoint of baking quality, it is obviously illogical to classify wheat solely on the basis of the percentage of infected kernels since one sample containing a low percentage of infected kernels may give poor bread, whereas another sample will produce good bread even though it contains a relatively large percentage of infected kernels. The extent of damage caused by any particular degree of infection depends upon a number of factors, such as the species of wheat-bug,

the stage of maturity at which the grain is attacked, the kind of wheat, and the original quality of the gluten.

It must be remembered that there are other insects which, like the wheat-bug, pierce the maturing kernels and produce pale areas and dark spots but which do not influence the quality of the gluten, dough, and bread. An example is the larvae of *Haplothrips tritici*, as recently reported by Grivanov (1938). We have examined a series of wheat samples which contained from 10 to 28% of infected kernels and yet had good gluten and made excellent bread; it is quite plain that the wheats were not punctured by harmful bugs.

It follows from the facts which have been cited that the only reliable method of estimating the quality of grain suspected of damage by the wheat-bug is to determine gluten or dough quality. At the present time such an estimation can be made most precisely with the help of Brabender's farinograph or Chopin's alveograph. As the disintegration of the dough takes place only after it has rested, it is necessary to record the farinograms and alveograms immediately after the dough is made and again after it has stood in a warm place for 60 to 90 min. A similar method of estimating the degree of grain infection by the wheat-bug, based on the use of Chopin's extensimeter, was adopted in France and legalized by a special regulation ("Office National Interprofessionnel du Blé," 1937). Price discounts for infected grain have been established in conformity with the degree of damage, called "virulence commerciale."

By what means is it possible to improve wheat damaged by the wheat-bug? In the studies of Berliner (1936), Kranz (1935), and others, there are numerous indications of the favorable effect of heat treatment on the quality of damaged wheat. We can fully confirm these indications; the heating of moistened grain with warm air gives good results, improving the quality of the gluten and the dough. Beneficial results are also obtained by adopting the method of Weickmann (1938) who used hot water. However, the most convenient and suitable method is the treatment of grain by steam. Upon treating wheat containing 32% of infected kernels with steam for 1.5–3.0 min, a normal gluten was obtained. It is presumed that the treatment of wheat with steam could be recommended not only for damaged grain but also as a simple method of conditioning normal wheat.

Summary

Studies of the biochemical properties of wheat which has been punctured by certain species of wheat-bugs show that such infection somewhat lowers the total nitrogen and considerably increases the

proportions of alcohol-soluble and water-soluble nitrogen, especially the latter. In wheat containing 10–15% of kernels punctured by the wheat-bug, the gliadin content of the gluten is substantially increased. Gliadin solutions prepared from badly damaged kernels are characterized by a lower viscosity and optical activity than normal; the gliadin has a higher total sulfur content, but the sulfhydryl compounds of the glutathione type are not increased.

Flour from infected wheat differs from normal in possessing higher titratable acidity, higher amylase activity, and a markedly greater proteolytic activity. Extensive proteolysis, with the accumulation of water-soluble nitrogen and of nitrogen not precipitable by trichloracetic acid, occurs during the autolysis of flour milled from infected wheat.

Damage caused by the wheat-bug, at least when the wheat is attacked during later stages of maturation, is localized in the region of the puncture. Endosperm dissected from the damaged areas of kernels is considerably lower in nitrogen content than that of the undamaged areas, and approximately one-half of the total nitrogen is water-soluble and not precipitable with trichloracetic acid solution. Damaged kernels contain an increased quantity of tyrosine, which serves as a rapid and convenient method of detecting the presence of infected kernels.

Proteinases of the infected wheat are most active in neutral or faintly alkaline solution. Their activity is greatly reduced by moderate acidification.

Water extracts of infected kernels influence the gluten of normal flour only slightly, whereas extracts obtained with 0.5% sodium carbonate solution rapidly destroy the gluten but lose their activity on heating. Dry enzyme preparations can be prepared from infected kernels; when 0.1% is added to normal flour, the gluten-forming properties are rapidly destroyed. Aqueous solutions of the dry enzyme preparations are completely inactivated by heating.

These studies prove that the destruction of gluten in infected wheat is caused by active proteolytic enzymes injected into the kernel by the wheat-bug. The harmful effect of an admixture of damaged flour or of enzyme preparations upon the quality of normal flours is much less for strong than for weak flours. It is therefore impossible to set a maximal allowable percentage of infected kernels which would be applicable to every type of wheat.

Normal gluten can be obtained from wheat containing up to 32% of infected kernels after treatment with steam for 1.5 to 3.0 min.

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pH VARIATIONS IN SODA CRACKERS AND THEIR CONTROL WITH DIAMMONIUM PHOSPHATE

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The maintenance of a uniform pH in soda crackers has long been a perplexing problem in the biscuit and cracker industry. Baking soda requirements may vary from day to day, and as yet no reliable method has been devised that will predetermine these variable requirements. Johnson and Bailey (1924) suggested a titration procedure for determining soda requirements, but this method was never widely accepted by the industry because it did not provide a complete solution to the problem. One technical shortcoming of this procedure was that it failed to control the variables occurring after the soda had been added at the dough make-up stage. These authors concluded that although a small excess of soda over that required for neutralization is desirable, crackers of excessive alkalinity often result if this excess is not accurately controlled. This was largely due, they believed, to the conversion of the excess bicarbonate to the more highly alkaline normal carbonate during baking. Micka (1941), in a later

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study of the soda requirements of cracker flours, concluded that the excess or "free" soda, as he called it, remained as bicarbonate during the baking, thus ruling out its conversion to the normal carbonate as a possible cause of increased alkalinity in soda crackers.

The investigation reported here was undertaken to determine the nature of pH variations in soda crackers and the possible control of these variations by means of chemical buffering agents.

Methods

The following experimental formula and procedure were used except where otherwise noted.

	Sponge	Dough
	g	g
Cracker flour	120	80
Yeast	1	—
Salt	—	2
Shortening	—	22
Baking soda	—	Variable
Water	50	23
Fermentation time	18 hours	5 hours
Fermentation temperature	80°F (26.7°C)	80°F (26.7°C)

To facilitate mixing and promote fermentation the sponges were made up on a 500-g flour basis and then subdivided at the dough make-up stage. Sponges were mixed in a McDuffee bowl only long enough to obtain a coherent dough, whereas the doughs were mixed 6 minutes at low speed, using the 3-quart bowl and the cake paddle of a Hobart mixer. After fermentation, the doughs were run through a sheeter three times, then cut, docked, and baked on a steel plate in a gas-fired oven at 500°F (260°C) for $4\frac{1}{2}$ minutes. All pH determinations were made electrometrically, using approximately 20 g of dough or 15 g of crackers with 100 ml of water. Doughs were ground with water, whereas the crackers were powdered before adding the water.

Results

The pH data presented in Figure 1 were obtained from a series of soda-cracker baking tests in which the quantity of bicarbonate had been varied from 0 to 0.8%. The shape of the curve shows that in a pH range between 6.75 and 8.75 very large pH increases were obtained with small increments of bicarbonate. This observation is of commercial significance because it is this range with which the manufacturer of soda crackers is concerned.

The magnitude of these pH variations is more strikingly shown in Figure 2 which presents data obtained on sponges and doughs, and on crackers in which the baking periods were varied. As the amount of bicarbonate is increased and the pH approaches the critical range, there

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is very little effect on pH during the baking. However, when the bicarbonate is increased sufficiently to bring the dough to pH 7.0 or slightly higher, a proportional increase in the pH of the dough results, but an abrupt shift towards greater alkalinity occurs during baking. This high pH persists with little change during a normal baking period of 4½ minutes, but tends to drop as the baking is extended. Thus there is a lowered alkalinity in overbaked crackers.

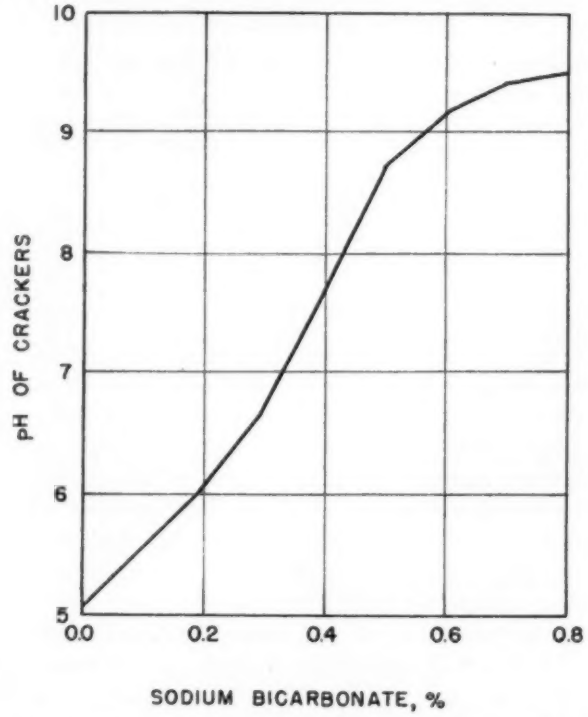

Fig. 1. Effect of increasing amounts of sodium bicarbonate on pH of soda crackers.

In further studies with varying baking periods, normal sodium carbonate and sodium hydroxide were used as neutralizing alkalies in such quantities as to provide the sodium-ion equivalent of the bicarbonate. The normal carbonate values for doughs and crackers, as shown in Figure 3, almost duplicated those of the bicarbonate. The sodium hydroxide curves in Figure 2 were considerably higher in pH immediately after dough make-up, but during dough fermentation they dropped to the approximate level of the bicarbonate doughs and then paralleled the pH of those doughs during the baking.

These studies were then extended to include tests with doughs and crackers containing excess amounts of the three alkalies: sodium bi-

carbonate, normal sodium carbonate, and sodium hydroxide. The same formula was used as before except that the yeast was increased to 1%, and a small amount of sodium sulfite (0.0125%) was added to facilitate handling of the doughs. The three alkalies were varied from none up to a sodium-ion equivalent of 2.5% sodium bicarbonate. A straight-dough procedure with a 2½ hour fermentation was used.

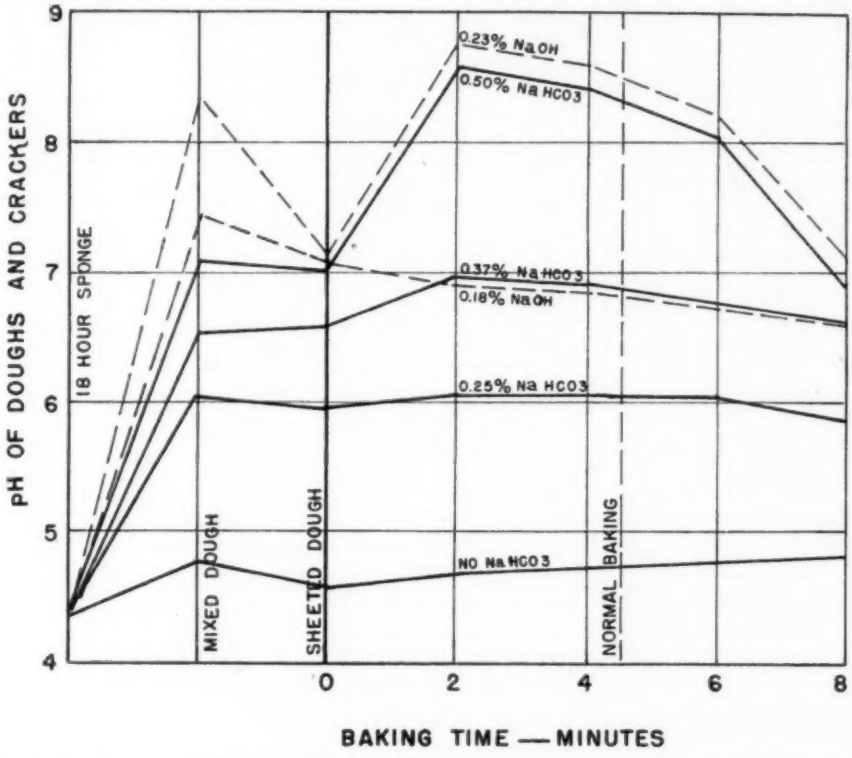

Fig. 2. Effect of increasing amounts of sodium bicarbonate and sodium hydroxide on pH of sponges, doughs, and crackers at variable baking periods.

The pH was followed in both the doughs and the baked crackers. The curves presented in Figure 4 show a wide spread in pH between the dough and cracker made with sodium bicarbonate and much smaller spreads when the normal carbonate and sodium hydroxide were used. Upon baking, the pH of the doughs containing carbonates increased whereas that of the doughs containing sodium hydroxide decreased. Each of the three alkalies tends to affect the pH of the dough in proportion to its specific alkalinity. However, in the baked cracker the pH of both the "bicarbonate" and "hydroxide" crackers tends to approximate that of the crackers made with normal carbonate.

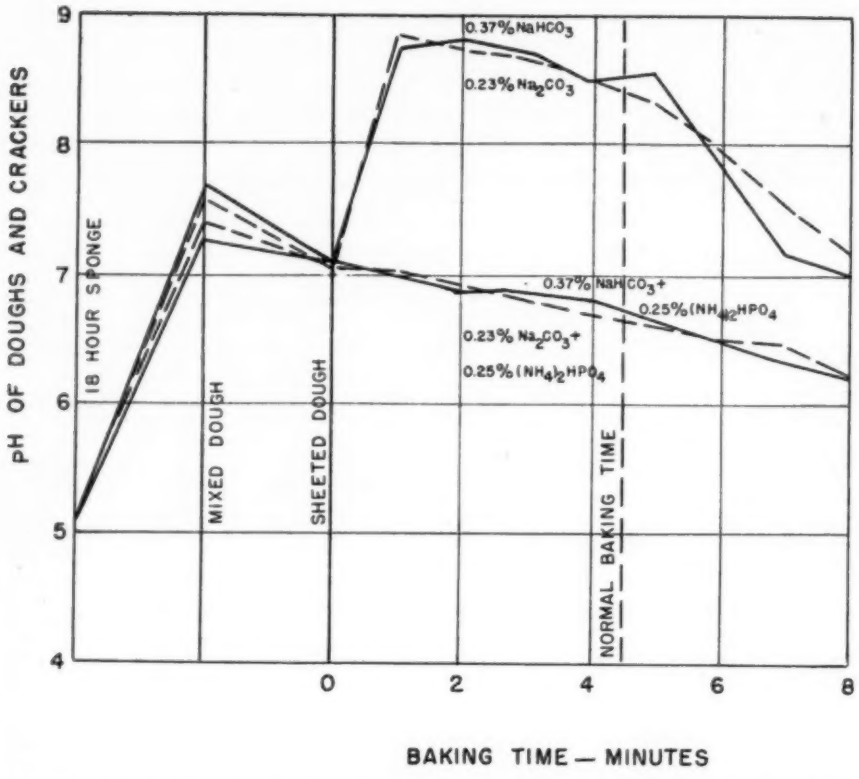

Fig. 3. Effect of diammonium phosphate in controlling pH of soda crackers at variable baking periods when using sodium bicarbonate or the normal carbonate as neutralizing agents.

It was of interest also to ascertain whether carbon dioxide remained in the crackers made with the largest amounts of the three alkalies. The analyses are given in Table I. The recovery of carbon dioxide

TABLE I

CARBON DIOXIDE IN FINELY PULVERIZED CRACKERS

Alkali used	Alkali added	Carbon dioxide evolved[1]	Approximate theoretical carbon dioxide recovery
	%	*ml*	%
Control	0.00	5.0	—
Sodium bicarbonate	2.50	21.5	25
Normal sodium carbonate	1.56	22.5	52
Sodium hydroxide	1.18	13.0	—
Control with added sodium bicarbonate[2]	2.50	71.0	100
Control with added normal sodium carbonate[2]	1.56	38.5	100

[1] Determined as outlined in Cereal Laboratory Methods (1941), p. 171.
[2] Powdered carbonates were weighed directly into the pulverized crumb of the control crackers before analysis.

from carbonates added directly to the pulverized cracker-crumb control was the basis for calculating the carbon dioxide which remained in the crackers containing the alkalies. These data suggest that the excess bicarbonate does not remain as such in the baked product.

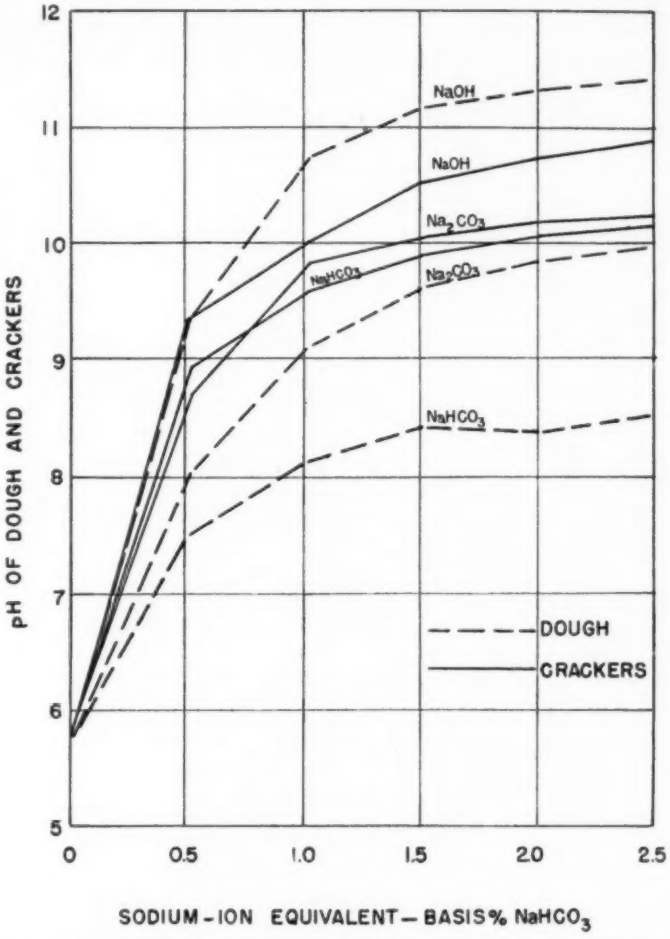

Fig. 4. Effect of excessive amounts of sodium bicarbonate, normal carbonate, and sodium hydroxide on pH of doughs and soda crackers.

However, the partial recovery of 52% of the carbon dioxide which was added to the dough as the highly alkaline normal carbonate, and the gain in the carbon dioxide content of crackers made from doughs which received sodium hydroxide, indicate that mass reactions may be involved in the baking-out of cracker doughs. These reactions probably involve the formation and breakdown of alkali-protein-acid complexes.

The commercial production of soda crackers has advanced to a high degree of efficiency. The various operations, such as mixing, fermentation, machining, are well controlled in most cracker plants, yet pH variations may occur and result in an inferior product. Any buffering agent which could be added to the dough in reasonable amounts and which would maintain the pH of the finished crackers within a desirable range would be of great value to the industry.

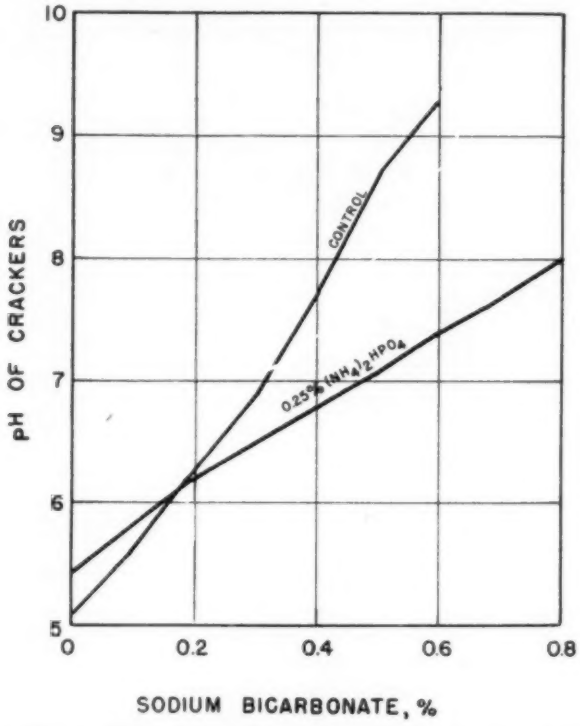

Fig. 5. Effect of diammonium phosphate in controlling pH of soda crackers made with increasing amounts of sodium bicarbonate.

Most of the common buffering salts, such as the acetates and phosphates of sodium and calcium, do not satisfactorily control the pH when used in practicable amounts. However, diammonium phosphate was found to be unusually effective. The addition of 0.25% diammonium phosphate to soda-cracker doughs in which the bicarbonate was used in increasing amounts held the pH of the crackers within relatively narrow limits (Fig. 5). Diammonium phosphate also prevents the alkaline shift which results from an excess of alkali over that required to neutralize cracker dough to pH 7.0 (Fig. 3). Furthermore, as shown in Figure 6, diammonium phosphate buffers on the

acid side. In general, however, acid crackers are not so troublesome in commercial production as are alkaline crackers.

The manner in which this ammonium salt tends to control the pH in soda crackers is unique. It is essentially a neutral salt and when mixed in a neutral dough apparentiy does not decompose to any appreciable extent. However, if excessive bicarbonate is present, the alkali formed during baking (Fig. 2) immediately tends to react with

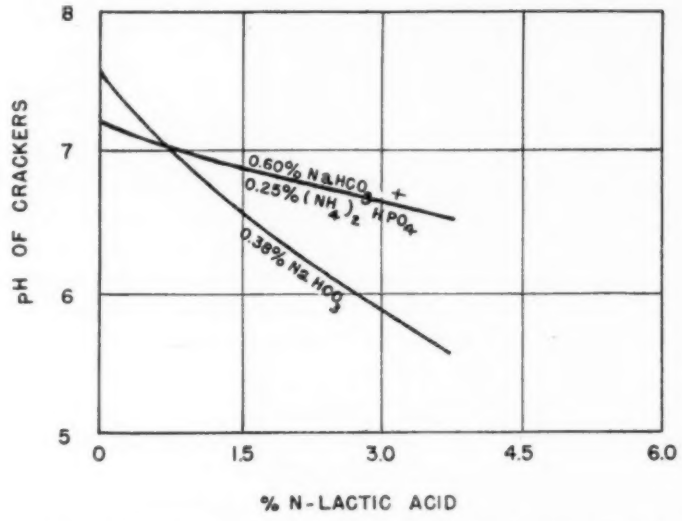

Fig. 6. Effect of diammonium phosphate in controlling pH of soda crackers on the acid side.

the diammonium phosphate, thereby causing ammonia to be driven off and phosphoric acid to be released; this acid, in turn, neutralizes the alkalinity. The sodium phosphates which remain as residual salts are well known for their buffering action. If, on the other hand, excess bicarbonate is not present, a shift to the alkaline side does not occur during baking, phosphoric acid is not released, and the diammonium phosphate apparently acts as a buffering salt. However, more complex reactions probably occur in which proteins and other dough constituents take part.

Practical Aspects

Considering the number of variables which influence the final pH of crackers, it is surprising that the average bakeshop superintendent controls this factor as effectively as he does.

During the 18-hour sponge fermentation, organic acids are formed which must be neutralized by the addition of sodium bicarbonate or "soda," as it is commonly known to the industry. As the average

plant lacks the facilities to determine the soda required by a procedure such as that proposed by Johnson and Bailey (1924), empirical methods nurtured within the industry have served as guides indicating the correct soda addition following long sponge fermentation. As an example, there is the anonymous advice to add 2 oz of soda per barrel of flour per degree Fahrenheit rise in temperature during this fermentation. Most experienced men can make a shrewd estimate of the amount of soda necessary to produce good crackers from the rise in temperature and "feel" of the sponge after 18 or 19 hours of fermentation. Trouble is sufficientiy common, however, to justify the dissemination of any information that might be helpful in maintaining a uniform pH from one batch of crackers to another.

The reasons for maintaining a uniform pH at some point between 7.2 and 8.4 are well set forth by Johnson and Bailey (1924). Proper texture, flavor, and appearance all depend largely upon accurate pH control. Commercially, soda crackers must be held within these pH limits if they are to be of acceptable quality and thus hold a strong place in their competitive field. The difficulties encountered in estimating the optimum addition of soda are indicated in Johnson and Bailey's observations that the soda requirements ranged from 7.75 lb to 9.75 lb of soda per 5 barrels of dough.

Factors involved in pH control from a practical standpoint include: flour characteristics, sponge formula, dough-trough conditions, temperature, humidity, fermentation time, and diammonium phosphate used as a buffer.

During recent years, flour for use in soda crackers has been adapted more closely to its particular function by a better understanding of the requirements and by methods of measuring the qualities desired. Today a survey of sponge and dough flours would probably show a greater difference in analysis between the average sponge flour and the average dough flour than existed 20 years ago. Terms such as "fermentation tolerance," "diastatic activity," and "maltose value" are all comprehended by the cracker baker when he observes that the "sponge rose high enough" and "feels normal." The influence of a flour from a well-blended wheat mix upon pH is usually fairly constant within a given shipment, but the experienced cracker baker notes a variation in pH when shifting from one brand of flour to another or from one shipment to another.

The proportion of flour to water in the sponge affects not only the pH but also the diastatic and proteolytic activity during fermentation. During the first 4 to 6 hours the rate of fermentation is slow; this is generally believed to be a period of hydration of the sponge constituents. The presence of more water in a "slack" sponge naturally

leads to a faster complete "wetting" and usually a more rapid drop in pH during the sponge fermentation. To keep this hydration period as uniform as possible, care should be taken to insure accurate measurement of the water and of mixing time.

One of the most difficult factors to control is the effect of the dough culture which remains on the inner surface of the dough troughs when they are emptied. This adhering dough can stimulate fermentation to such an extent that 10 to 12 oz more soda per 5 barrels of dough are required, even when all other factors are held as uniform as possible. This difference is between the first dough mixed in a trough that has been scraped clean and out of use for two or three days, during which time the culture loses its vitality, and a second mix in the same trough immediately after it has been emptied. In the latter trough, the third mix will require 4 ounces more of soda, and the fourth and fifth mixes will each require additional 2-oz increments. These data are based upon the use of metal troughs. It is the prevailing opinion within the industry that wooden troughs do not show quite such wide variations as metal troughs in the soda requirements for successive batches.

Temperature is the cracker baker's measuring stick. It indicates the point at which fermentation will start and, to a limited extent, when it will be complete. For example, a sponge "set" at 72°F (22.2°C) and fermented in a room held at 81°F (27.2°C) will give a temperature reading of 83°F (28.3°C) after 18 hours. Assuming that 7 lb of soda will give the desired pH of 7.8 in the resulting crackers, how much soda will be required by the next sponge that gives a reading of 82°F (27.8°C) at the end of the fermentation period? Data which the authors have accumulated indicate that, within the range of 8 to 12 degrees rise in temperature during the sponge fermentation, 6 oz of additional soda per 5 barrels of flour are required per degree Fahrenheit rise in temperature; no data are available beyond this temperature range.

In studying the control of the dough-room factors it has been found that 81°F (27.2°C) and 76% relative humidity provide a favorable environment for fermentation. A lower temperature may hinder fermentation and proteolytic activity to such an extent that the crackers will have a pH between 9 and 10 and exhibit a tendency to "buckle" out of shape during the bake. If the relative humidity decreases sufficiently to allow the doughs to crust over during the 3- or 4-hour fermentation after the soda has been added, the consequent physical "holding down" of the dough seems to have much the same effect as a slight drop in temperature.

On the other hand, if a little extra time elapses before baking, the crackers will drop below pH 7.0 with all the attendant ills of "acid" crackers. This undesirable result emphasizes the importance of scheduling plant operations so that the time interval between the setting of the successive sponges equals the baking time of each dough.

To illustrate these factors, assume a dough room at 81°±1°F and 76%±2% relative humidity. A series of five sponges are set in troughs that were scraped and out of use for three days. The soda requirement under these conditions is 7 lb per 5 barrels for 11°F rise in temperature with a 6-oz differential per degree variation. Doughs 1 and 2 were set at 72°F (22.2°C) and rose to 82°F (27.8°C) and received 6 lb 10 oz of soda apiece. Doughs 3 and 4 were set at 73°F (22.8°C) and rose to 84°F (28.9°C) requiring 7 lb of soda apiece. Dough 5 was set at 71°F (21.7°C) and rose to 83°F (28.3°C) and therefore called for 7 lb 6 oz of soda. The same troughs were used as soon as emptied, and the next day the soda schedule was built around a basis of 7 lb 10 oz of soda per 5 barrels per 11-degree rise with a 6-oz differential per degree variation. To follow through, the third mix would be based upon 7 lb 14 oz of soda, the fourth mix upon 8 lb, and the fifth mix upon 8 lb 2 oz.

Despite precautions and control measures, the vagaries of fermentation are likely to cause trouble. However, these potential snags can be forestalled in large measure by the use of diammonium phosphate as a buffer during the baking process. This versatile chemical tends to bring both acid and too-alkaline crackers back into the acceptable range. The addition of 2 lb of diammonium phosphate and 2 lb of soda above the schedule requirements makes it possible to produce crackers day after day in which the pH will vary only 0.2 or 0.3.

Observations in cases where the dough-room conditions varied from the standards set up indicate that the buffer action is valuable in combating the visible effects of an excess of residual soda. Doughs in which the fermentation is retarded by a low temperature usually have enough residual soda to give the baked crackers a dark brown color or "bloom" associated with a pH at some point above 8.4. This color reaction seems to be much less marked when diammonium phosphate has been added to the dough, even in cases where the excess of soda is sufficient to overcome the buffer action and result in a pH above 8.4.

Summary

The pH variations encountered in the fermentation and baking of soda crackers have been investigated. When sodium bicarbonate is added in amounts just sufficient to neutralize dough ingredients and acids of fermentation, little or no change in pH occurs during baking.

However, when an excess of bicarbonate above that required for neutralization is added to the dough, a large shift in pH toward the alkaline side occurs upon baking. Diammonium phosphate was found to be unusually effective in preventing these pH shifts. It also buffers crackers on the acid side of neutrality.

Evidence is presented that excessive bicarbonate in a soda cracker dough does not remain in that form during the baking of the cracker. Conversion to the normal carbonate, along with more complex reactions involving proteins and other dough constituents, undoubtedly takes place.

Problems involved in controlling pH in the commercial production of soda crackers are discussed.

Acknowledgments

Grateful acknowledgment is made to Mr. Thomas Hollingshead, Director, Technical Institute, Independent Biscuit Manufacturers Company, Inc., 7002 North Clark Street, Chicago, Illinois, for valuable assistance in planning the investigation; also the Schulze-Burch Baking Company, 1133 West 35th Street, Chicago, Illinois, for their helpful cooperation.

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THE SHORTENER TOLERANCE OF BISCUIT AND SELF-RISING FLOURS

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(Presented at the Annual Meeting, May 1942; received for publication May 18, 1942)

Several years ago, the Cincinnati section, American Association of Cereal Chemists, appointed a Research Committee to further activity in soft wheat problems. The project which was selected comprised a study of the optimum shortener level in biscuit flours of varying viscosity. This paper presents the studies which were undertaken.

Whiting (1932) used 16% shortening in the work of the A.A.C.C. Subcommittee on Tests for Biscuits and Self-Rising Flours. He suggested that the shortening levels in many biscuit formulas may be too low for optimum results. Walter (1933) employed 13% shortening for soft flours and 15% for hard flours. In more recent studies (Whiting, 1936; Gookins, 1941), shortener levels of the order of 12.5% have been advocated for all biscuit-type flours. In Cereal Laboratory Methods

(4th ed., 1941), 12.5% shortening is specified in the biscuit test for self-rising flours.

The objects of the present study were to determine whether the 12.5% shortening level recommended in test-biscuit formulas is too low and whether the shortener requirements vary with the protein content and viscosity of the flour. Three series of collaborative studies were undertaken, the first in 1936–37, the second in 1939–40, and the third in 1940–41. In the first series, biscuits were baked from three bleached, soft wheat flours selected to cover the viscosity range encountered in commercial self-rising flours, employing corn oil as the shortening agent at levels of 4½ to 17½% in 2% increments. The results of these tests were not sufficiently conclusive to show definitely whether shortener requirements vary with the viscosity of the flour. The strongest flour, however, did not exhibit as much tolerance to variations in shortening level as the two weaker flours. It was the consensus of opinion that the study should be repeated, employing still higher percentages of shortening and adjusting the absorption to compensate for the effects of the varying fat levels on the consistency of the dough.

These suggestions were followed by the 1939–40 Research Committee. Only the second and third series of studies will be detailed here.

Materials and Methods

Second Series (1939–40 Research Committee). Two flours, D and E, which differed widely in protein content and viscosity (Table I) were converted to self-rising flours by the addition of 1.86% monocalcium phosphate, 1.5% powdered sodium bicarbonate, and 1.75% salt. Summer-grade cottonseed oil which had been alkali-refined, deodorized, and lightly bleached was employed as the shortening agent at levels of 10, 15, 20, and 25% (flour basis). Preliminary tests were made to determine the optimum absorptions for each fat level for both flours. Detailed instructions for carrying out the biscuit test were supplied each collaborator. The required amount of water was placed in the mixing bowl, the flour added, and the desired amount of oil distributed over the surface of the flour. Mixing was conducted with a Kitchen Aid mixer for 10 seconds at low speed, the mass cut down, and the mixing continued for an additional 10 seconds at low speed. In other details the instructions essentially corresponded to those now given for the biscuit test in Cereal Laboratory Methods (4th ed., 1941). Biscuit volume was determined by seed displacement. The biscuits were judged for grain, tenderness, flavor, and crumb color, according to the scoring system proposed by Walter (1935) and subsequently

included in the latest revision of Cereal Laboratory Methods (4th ed., 1941). Total scores, which include biscuit volume in addition to the quality factors just enumerated, were computed by each laboratory. Since the adverse effect of increased shortening on crumb color would influence the total scores, the total scores omitting crumb color were also computed to obtain a more reliable measure of the effect of shortening level on the other quality factors.

Third Series (1940–41 Research Committee). Two shortenings, a lightly bleached cottonseed oil and hydrogenated shortener, each at levels of 13, 15, 17, and 19%, were investigated employing one flour (F, Table I). As in the previous year, the optimum absorption for each fat level was determined by preliminary experiments. For each 1% increase in shortener, a decrease of about 0.4% water was necessary. The method of incorporating the hydrogenated shortener was the same as that given in Cereal Laboratory Methods (4th ed., 1941). In all other respects the experiments were conducted by the methods outlined for the second series.

TABLE I

CHARACTERISTICS OF FLOURS EMPLOYED IN BISCUIT TESTS

Flour [1]	Protein [2]	Ash [2]	pH	Viscosity	
				No time	1-hr. digestion
	%	%		° *Macm*	° *Macm*
D	10.8	0.36	5.7	148	164
E	8.0	0.40	5.9	57	72
F	8.5	0.37	5.2	61	84

[1] Flour D: 92% patent, bleached with Agene and Novadel, milled from a mixture of 30% Illinois hard, 20% low protein Kansas, and 50% Indiana red wheats.
Flour E: 92% patent, bleached with Agene and Novadel, milled from Indiana red wheat.
Flour F: 85% patent, bleached with Beta Chlora and Novadel, milled from Indiana red wheat.
[2] 15.0% moisture basis.

Results and Discussion

The 1939–40 studies are summarized in Figure 1, which shows the average biscuit scores for all collaborators for the various shortening levels. At any given shortener level, the softer flour (E) gave the better biscuits. For this flour the optimum shortening level was 15% as compared with 20% for the stronger flour (D). For all practical purposes, however, there is little difference between the optimum shortening level of the two flours since the quality of the biscuits made from the weak flour with 15 and 20% shortening, respectively, did not differ materially. Above 20% shortening, a marked decrease in biscuit

quality occurred with both flours. In Figure 1 the total scores, less crumb color, are also shown. It is noteworthy that increasing additions of the cottonseed oil, which was appreciably pigmented, had a more pronounced effect on crumb color than on the other quality factors.

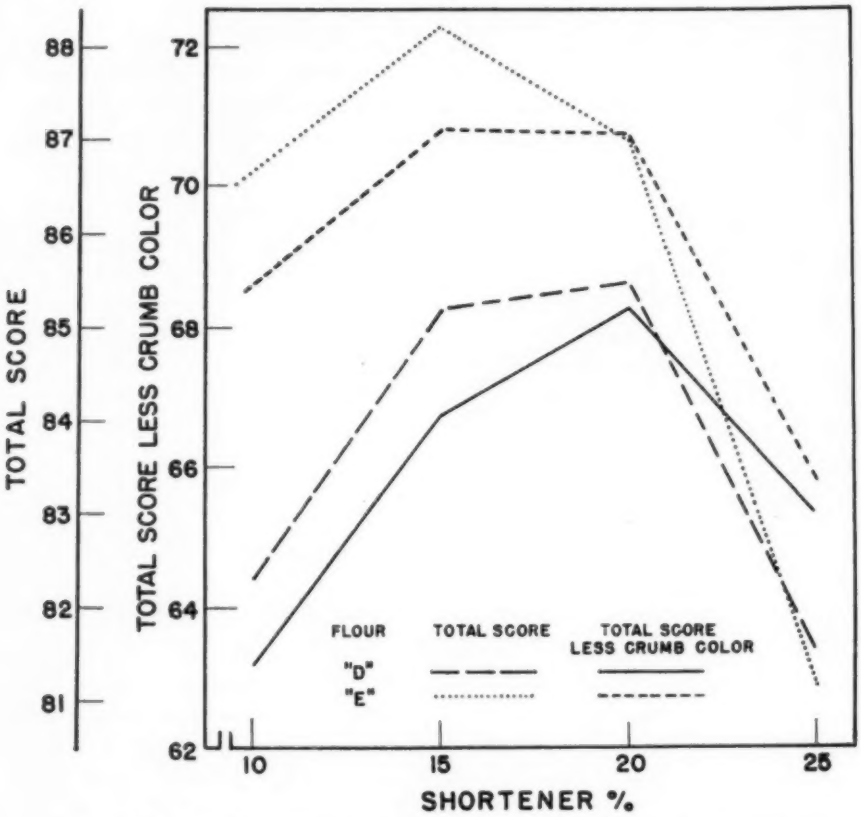

Fig. 1. Effect of varying percentages of cottonseed oil shortener on total score of biscuits from Flours D and E (outer ordinate). The effect of crumb color on total score is also removed for comparative purposes (inner ordinate). (1939–40 Research Committee, Cincinnati Section, A.A.C.C.)

The results of the 1940–41 studies are summarized in Figure 2. The liquid and solid shorteners both gave biscuits of optimum quality when used at the 19% level. This level is in close agreement with that found in the previous year's work.

The optimum percentage of shortening found in these studies is considerably higher than the 12.5% level currently employed in biscuit tests. As the fat content was increased, the specific volume of the biscuits showed little change, but the tenderness of the biscuits was markedly improved; also, crumb grain was slightly better. Although

cottonseed oil had a significant effect upon the crumb color of the biscuits, the decrease in crumb color scores with increasing shortening content of the biscuits was not sufficient to offset the over-all improvement in the other quality factors. This is shown by Figure 2, as the

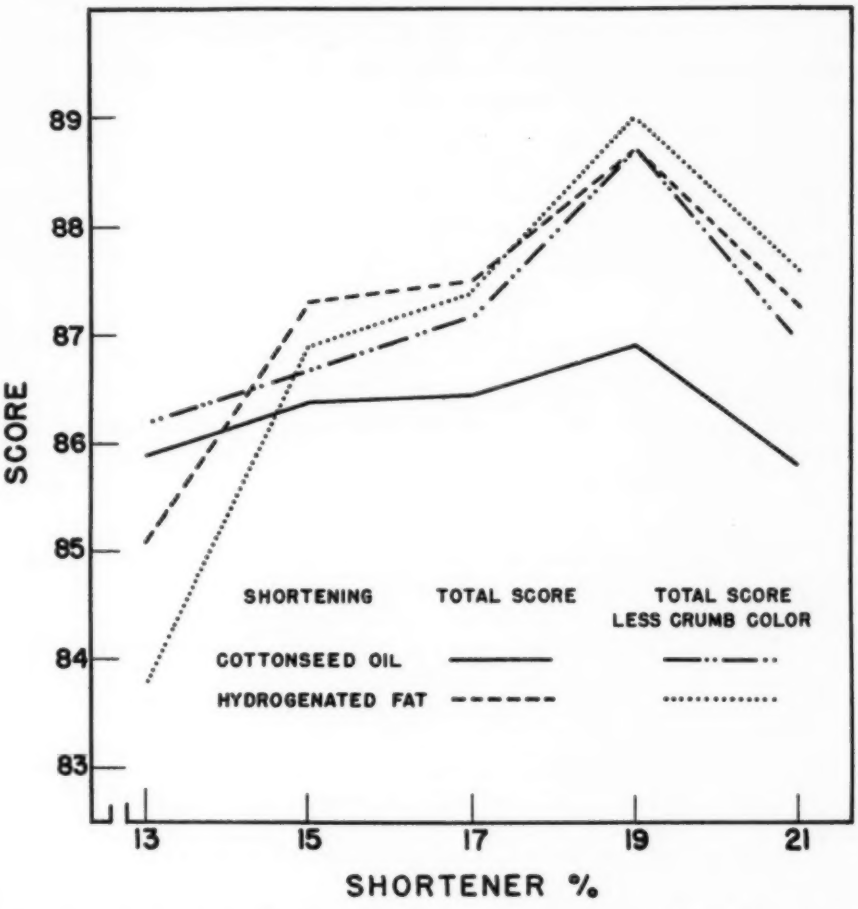

Fig. 2. Effect of varying percentages of cottonseed oil and hydrogenated fat on total score of biscuits. Scores are also shown exclusive of crumb color; these are adjusted for the effect of crumb color on the total score. (1940–41 Research Committee, Cincinnati Section, A.A.C.C.)

cottonseed oil curve (adjusted for the effect of crumb color on the total score) practically coincides with both the adjusted and unadjusted curves for the hydrogenated fat.

Summary

The optimum shortener level for flours of the type generally supplied for biscuit-making purposes is approximately 19%. Commonly used

test-biscuit formulas specify 12.5% shortening. In employing this high shortening level, the absorption must be decreased to compensate for the effect of the extra fat on dough mobility; a decrease in water absorption of about 0.4% is required for every 1% increase in shortener.

Comparative biscuit tests with a liquid (cottonseed oil), and a solid shortener (hydrogenated shortening) indicated that the optimum shortening level is independent of the type of shortener.

Biscuit flours of varying strength, as indicated by protein content and viscosity, did not differ materially either in their tolerance to varying percentages of shortening or in the quantity required to yield optimum biscuits. However, the softer flours gave the superior biscuits and maximum biscuit quality was obtained with slightly lower percentages of shortening than are needed with the stronger flours.

Acknowledgments

The authors acknowledge with gratefulness the services of the following committeemen: L. W. Baker, L. Brown (deceased), C. F. Evert, J. R. Hedding, E. G. Kornreich, J. S. Kelley, Clifford Nelson, C. O. Oppen, J. A. Shellenberger, H. M. Simmons, E. L. Ulrey, Donald Wade, and R. W. Whiteside.

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THE SULFHYDRYL GROUPS OF WHEAT FLOUR

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(Received for publication February 5, 1943)

In previous studies of the effects of oxidizing agents on fermenting doughs (Sullivan, Howe, Schmalz, and Astleford, 1940), the need for a study of the sulfhydryl groups of the protein and other fractions of wheat flour has been expressed. The following work was done to help fill that need.

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In a study of the sulfhydryl groups of proteins it should be kept in mind that these may exist in at least two well-recognized forms. Some proteins, such as urease, contain free or reactive sulfhydryl or —SH groups. These groups cause the protein to give a positive nitroprusside test when it is in the native form. In some proteins, such as egg albumin, however, there are no free sulfhydryl groups, but after denaturation these groups seem to be liberated so that they give a positive nitroprusside test. These groups are designated as bound or unreactive sulfhydryl groups.

It has been postulated that the improving effects of oxidizing on a dough are due to the oxidation of the sulfhydryl groups of the flour proteins. This oxidation would result in the formation of disulfide cross-linkages between protein molecules. This increased degree of polymerization would therefore seem to produce a dough of greater coherence and gas-retaining properties.

Balls and Hale (1940) have shown the presence of reactive sulfhydryl groups in the petroleum ether extract of flour. Sullivan, Howe, and Schmalz (1936) have demonstrated the presence of glutathione, a tripeptide containing cysteine, in wheat germ. Small amounts of this sulfhydryl-bearing material are undoubtedly to be found in flour as a result of incomplete germ separation during milling.

To our knowledge, no conclusive evidence of the presence of either reactive or unreactive sulfhydryl groups in the proteins of wheat flour has been presented.

Methods

The presence of reactive sulfhydryl groups in wheat flour proteins was indicated by two tests. The nitroprusside test was made by placing a small amount (approximately 10 mg) of the material on a porcelain spot plate and adding one drop of 1 : 1 ammonium hydroxide and one drop of freshly prepared 5% sodium nitroprusside solution. The development of a pink color on the surface of the material within 30 seconds was taken as an indication of the presence of reactive sulfhydryl groups in the material being tested.

Another more sensitive but less specific test was developed from the work of Anson (1941). He showed that cysteine was the only amino acid which reduced potassium ferricyanide immediately and stoichiometrically in dilute solution at neutral pH. The ferrocyanide formed by this reduction was then converted to Prussian blue which was readily discernible and could be, if desirable, measured quantitatively.

In making this test, 2 ml of the protein dispersion (approximately 2%) was treated with 0.2 ml of 1*M* phosphate buffer (pH 6.8) and

1.0 ml of 0.01*M* potassium ferricyanide. After proceeding for 5 minutes, the reaction was halted by the addition of 0.5 ml of 2*N* sulfuric acid. Then 0.1 ml of the ferric sulfate reagent described by Folin and Malmros (1929) was added. The development of a blue color in the solution was taken as an indication of a reduction of part of the ferricyanide by the protein dispersion, and, in the testing of purified proteins, indicated the presence of reactive sulfhydryl groups. This color could also be measured colorimetrically and converted to its cysteine equivalent from a calibration curve.

To test for the presence of unreactive sulfhydryl groups, the above two tests were applied to the protein material after it had been subjected to treatment intended to denature it.

The nitroprusside test was applied as above after treatment of the proteins with heat, guanidine hydrochloride, or urea. Approximately 10 mg of crystalline guanidine hydrochloride or 15 mg of urea were used in each test.

The ferricyanide test was applied after the addition of 0.5 ml of 10% Duponol PC solution. This is a monosodium sulfate of mixed fatty acids, chiefly C_{10} to C_{18}, a synthetic detergent capable of denaturing egg albumin. This test was also applied after heating the protein dispersions both in the presence and absence of this amount of the detergent. Uniformly positive tests in all of these cases were taken as indications of the presence of unreactive sulfhydryl groups in that particular protein.

The Gluten Proteins

Gluten was prepared from freshly milled unbleached patent flour. The gluten was washed from a flour-water dough, dispersed in 0.005*N* acetic acid, precipitated by the addition of phosphate buffer (pH 6.8) to 0.05*M*. This purified gluten was again dispersed in 0.005*N* acetic acid. Both the dispersion and a sample of the precipitated gluten gave a negative nitroprusside test. The dispersion gave a negative test for the presence of free reducing groups by the ferricyanide technique. From this it was indicated that the gluten proteins did not contain any free sulfhydryl groups.

To test for the presence of any unreactive groups which might be liberated and become reactive during denaturation, the following procedures were employed. Samples of precipitated gluten were heated, treated with guanidine hydrochloride and with urea, and tested with nitroprusside. These tests were uniformly negative. Portions of the gluten dispersion were boiled in the presence and absence of Duponol PC and also treated with Duponol PC at room temperatures. When these dispersions were tested by the Anson ferricyanide technique de-

scribed above, the results were also negative. From this it was concluded that the gluten proteins did not contain unreactive sulfhydryl groups.

Sullivan, Howe, Schmalz, and Astleford (1940) showed that a solution of 2,6 dichlorophenol-indophenol is eventually decolorized when boiled in the presence of gluten. They interpreted this as indicating a reduction of the 2,6 dichlorophenol-indophenol by sulfhydryl groups. Todrick and Walker (1937) employed a similar technique for the estimation of the sulfhydryl groups of egg albumin and claimed it to be specific for those groups.

The results of Sullivan, Howe, Schmalz, and Astleford (1940) were confirmed. It was also found that at boiling temperatures gluten

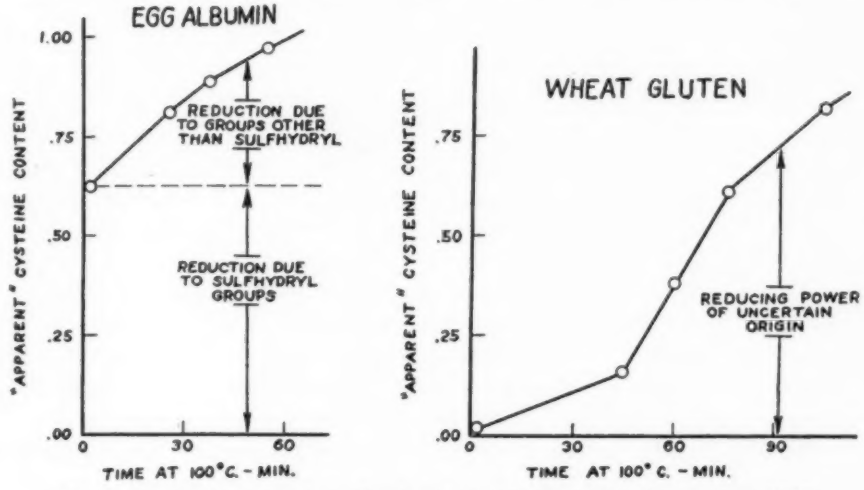

Fig. 1. The reducing power of egg albumin and wheat gluten as a function of time at 100°C.

reduced ferricyanide under the conditions described above. Anson (1939) showed that groups other than the sulfhydryl of egg albumin were able to reduce ferricyanide at boiling temperatures. A sample of egg albumin was prepared after the method of Sorenson with the exception that no attempt was made to obtain a crystalline product. The final solution was dialyzed against distilled water until free of ammonium sulfate. The sulfhydryl content of this protein was determined and found to be equivalent to 0.62% cysteine. Aliquots of this solution and of the gluten dispersion were subjected to the action of ferricyanide in the presence of Duponol PC for various periods at boiling temperatures. The amount of ferricyanide reduced was then estimated colorimetrically and expressed as "apparent cysteine content" of the respective proteins. These results are presented graphically in Figure 1.

While these results alone do not necessarily disprove that the reducing ability of gluten at boiling temperatures is due to sulfhydryl groups, they do support the belief that the action is due to other groups.

The Water-soluble Proteins

The soluble proteins were isolated from freshly milled unbleached patent flour. One hundred g of flour were digested with 200 ml of distilled water. The dispersion was centrifuged with a Sharples Super Centrifuge. The supernatant liquid was saturated with solid ammonium sulfate and the precipitated proteins separated by filtration. These proteins were redispersed and reprecipitated twice more. The precipitated proteins gave a faintly positive nitroprusside test while in the native state. When dispersed they were able to reduce ferricyanide even without the addition of Duponol PC. These results seemed to indicate the presence of reactive sulfhydryl groups in the soluble proteins. When these proteins were precipitated by the addition of four volumes of alcohol and then redispersed, however, they gave negative responses to both tests. When the filtrate from which these proteins had been precipitated was evaporated to dryness there remained a slight residue of oily material which gave a positive nitroprusside test.

The following experiment was performed to investigate this phenomenon more fully. The soluble proteins were isolated from 1200 g of flour as described above by precipitation from saturated ammonium sulfate. The precipitate was washed with ethyl alcohol and petroleum ether and the washings evaporated under vacuum. The residue consisted of 2 g of fatty material. The nitrogen content of this material as well as the fraction insoluble in acetone is shown in Table I along

TABLE I

Analysis of Fatty Material Isolated from the Soluble Proteins

Determination	Isolated fraction	Flour fat[1]	Phosphatide[1]
Kjeldahl, % *N*	1.65	1.65	4.28
Precipitated from acetone, %	20.4	25.0	100.0

[1] Values reported by Sullivan, *et al* (1940).

with similar values reported by Sullivan, *et al* (1940) on representative flour fat and on the phosphatide fraction of flour fat. These analyses indicated that this fraction was similar to whole flour fat and was not especially rich in phosphatides.

That the sulfhydryl reactions of these soluble proteins were due to the presence of contaminating lipids was further confirmed when the

proteins prepared from ether extracted flours failed to give positive nitroprusside or ferricyanide reactions without preliminary denaturation.

After the extraction of lipid material the soluble proteins were dispersed in a small amount of water to give a thick viscous paste. After treatment with guanidine hydrochloride or urea they gave a positive nitroprusside reaction. After treatment with Duponol PC these proteins reduced ferricyanide under the conditions specified above. From these reactions it was concluded that the soluble proteins of wheat flour contained unreactive sulfhydryl groups which were made reactive by denaturation.

Summary

A preliminary study of the sulfhydryl groups of the various fractions of wheat flour revealed no indications of the presence of either reactive or unreactive sulfhydryl groups in the gluten proteins.

There appeared to be no free sulfhydryl groups in the soluble protein fraction of flour. However, there were definite indications of the presence of unreactive sulfhydrvl groups which were liberated on denaturation of these soluble proteins.

The presence of free sulfhydryl groups in the lipid fraction, first demonstrated by Balls and Hale, was confirmed.

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VARIETAL, STATION, AND SEASONAL EFFECTS UPON SOME PROPERTIES OF MIXOGRAMS MADE FROM HARD RED SPRING WHEAT FLOURS BY VARIOUS MIXING METHODS [1]

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In evaluating the quality of wheat varieties it is exceedingly important that new varieties be compared with well-known wheats acceptable to the milling industry. It is evident that the greater the number of different quality comparisons made, within reasonable limits, the better will be the chances of finding an improved variety. Mixograms furnish an entirely different basis for judging wheat quality from that of protein content, loaf volume, ash, etc., and appear to supply valuable supplemental information regarding that elusive property of wheat known as "quality."

As no recent review is available, it is advisable to summarize those mixograph investigations that deal directly with wheat variety evaluation. Swanson (1939) postulated that the mixogram properties most closely related to baking results were: steepness or ascending slope of the curve; dough development time or time to reach the peak; the character of the peak, whether sharp or rounded; and the general width and height of the curve. It was pointed out that the mixogram characteristics of different wheat varieties should not be compared unless the wheats are grown under comparable environmental conditions and have approximately the same protein content. Varietal patterns were clearly visible among the curves obtained. Varietal differences have likewise been noted by Larmour, Working, and Ofelt (1939) and Ofelt and Sandstedt (1941).

Sandstedt and Ofelt (1940) thought that both baking and mixograph studies made at a uniform protein level were more informative than if made at the original flour protein content. The effect of protein content on curve height was pointed out. Swanson (1940) also commented on the effect of protein content on curve height and on the ascending and descending slopes of the curve. He later (1941) showed that dilution of flour with starch to a common protein level reduced the curves within a variety to the same pattern, but did not obscure the characteristic differences between varieties. The effect of water absorption upon height was found to be less than that of protein content.

[1] Published with the approval of the Director of the Experiment Station.

Johnson, Swanson, and Bayfield (1943) thought the greatest value of mixogram data lay in supplementing baking results. Relative mixing requirements, mixing tolerance, and varietal curve properties, could be derived from the mixograms. Significant relations between protein content and various mixogram characteristics were found. These researchers concluded that protein content was a more likely index of loaf volume than any curve property considered in the study. Swanson and Andrews (1943) stated that the varietal pattern of mixograms was, for all practical purposes, determined by the gluten protein and not by the starch.

Descriptions of mixogram diagrams, with indicated methods of measurement of the more important characteristics, were published by Swanson and Johnson (1943) and by Harris, Sibbitt, and Banasik (1943).

The investigations to be described were undertaken to determine the influence of certain factors on stage of dough development, range of stability, and curve height; they are, in general, a continuation of the work reported by Harris, Sibbitt, and Banasik (1943). The specific objectives were (1) to ascertain if significant differences in mixogram properties exist between varieties and between environments, and (2) to ascertain whether varietal and station differences are inherent in the gluten or starch component of the wheat. The effect of mixing method was also studied.

Materials and Methods

In the first series, 32 straight grade experimental flours milled from eight varieties (see Table II) of hard red spring wheat, grown under comparable conditions at four stations in North Dakota in 1942, were investigated. A second series represented six of the varieties of series one from the same four stations, but grown in 1941 and 1942.

The milling was done on an Allis-Chalmers mill in a room held at approximately 70°F and 65% relative humidity. A National micro-recording mixer (mixograph) was used; its head had a speed of 89 rpm, and the spring setting was 9. The apparatus was enclosed in an air-conditioned case held at 30°C ± 3° and 80% relative humidity.

Flours were mixed with distilled water or with the ingredients of the malt-phosphate-bromate formula. The required quantity of liquid, as determined by an experienced operator when baking the flour, was added to 25 g of flour (13.5% moisture basis), and the mixer was run from 8 to 10 minutes, depending upon the variety under test. In using fermented doughs, 50 g of flour was mixed with the malt-phosphate-bromate ingredients in the Hobart equipped with special dough hooks. The doughs were then divided into equal portions by

weight and fermented for 3 hours at 30°C and 75% relative humidity. Punches were made after 105 and 155 minutes of fermentation, respectively. After 3 hours one dough was taken from the fermentation vessel, placed in the mixograph bowl, and a 5-minute curve made, while the other was baked.

The starch used for diluting the flours to a constant protein level, and the dried gluten used in the gluten and starch blends, were prepared by the procedure described by Harris and Sibbitt (1941). The flours were diluted with starch to a protein level of 12%, while the gluten

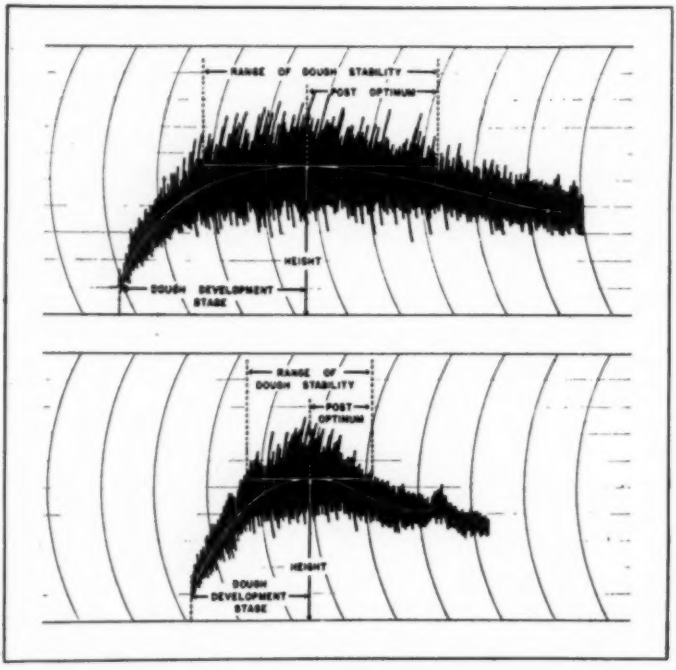

Fig. 1. Representation of the method used in securing the mixogram property measurements. Upper curve was made from a flour with long mixing requirements and range of dough stability. Lower curve was from a flour having an exceptionally short dough development stage and poor dough stability.

and starch blends were at a level of 13.5% (13.5% moisture basis). In the present work gluten-starch blends signify different glutens blended with a constant starch substrate, while conversely starch-gluten blends were made with variable starches blended with a constant gluten base. Moisture content was determined by drying in an electric oven at 130°C ± 2° for 1 hour. The protein was found by the Kjeldahl-Gunning method using 1 g of flour.

Figure 1 shows the method followed in securing the measurements which are similar to those described by Harris (1942). "Dough de-

velopment stage" may be somewhat of a misnomer for remixed doughs following fermentation, but it has been used to avoid confusion.

Results and Discussion

The influence of several factors on mixogram properties is shown in Table I. The malt-phosphate-bromate formula lengthened the dough development stage and possibly increased the curve height, while fermentation markedly reduced the length of the dough de-

TABLE I

MEAN VALUES OF MIXOGRAM PROPERTIES FROM THE VARIOUS SERIES OF DETERMINATIONS MADE ON THE DIFFERENT SAMPLES

Description of series	Dough development stage	Range of stability	Post-optimum stability	Curve height
	cm	*cm*	*cm*	*cm*
Flours, original protein content, flour-water formula	4.59	4.97		7.32
Flours, 12% protein level, flour-water formula, no fermentation	4.53	5.07	2.56	7.01
Flours, 12% protein level, malt-phosphate-bromate formula, no fermentation	6.39	5.04	2.76	7.86
Flours, 12% protein level, malt-phosphate-bromate formula, fermented and remixed	2.89	3.89	1.82	8.15
Flours, 12% protein level, flour-water formula, no fermentation				
1941 crop	5.17	3.86		5.46
1942 crop	5.11	4.07		5.93
Gluten-starch blends, malt-phosphate-bromate formula, no fermentation	4.13	4.54	2.69	8.47
Gluten-starch blends, malt-phosphate-bromate formula, fermented and remixed	2.65	3.46	1.87	8.71
Starch-gluten blends, malt-phosphate-bromate formula, no fermentation	5.06	4.38		8.11
Starch-gluten blends, malt-phosphate-bromate formula, fermented and remixed	3.35	3.49		8.60

Note: Thirty-two samples were used in all comparisons except for between years, where 24 were employed.

velopment stage and range of stability, and apparently tended to increase curve height. Little difference is apparent in the results between the flours of the 1941 and 1942 crops. In both of the gluten-starch blends, fermentation reduced dough development stage and range of stability in the same manner as for the original flours.

Table II presents the varietal averages of the four mixogram properties from the diluted flour and gluten-starch blend doughs of the 1942 crop. Differences are apparent among varieties in dough

TABLE II

Comparative Varietal Averages of Mixogram Properties from Flour and Gluten-Starch Blend Unfermented Doughs Made with Malt-Phosphate-Bromate Formula

(Arranged in increasing order of flour dough development stage)

Variety	Dough development stage		Range of stability				Curve height	
			Total		Post-optimum		Flour [1]	Blend [2]
	Flour [1]	Blend [2]	Flour [1]	Blend [2]	Flour [1]	Blend [2]		
	cm	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*
2822	2.99	2.23	3.31	2.60	1.65	1.32	8.05	8.59
Pilot B	3.56	2.65	3.64	3.33	1.71	1.76	8.08	8.73
Renown	3.99	2.69	3.55	3.41	1.69	1.86	8.04	8.59
Regent	4.33	3.03	4.45	3.90	2.28	2.18	7.89	8.54
2829	4.64	3.33	4.39	3.83	2.01	2.30	7.73	8.25
Thatcher	4.90	4.29	4.44	4.45	2.35	2.66	8.10	8.55
Rival	5.55	4.26	5.44	4.85	3.01	2.82	8.18	8.68
Vesta	7.23	4.64	6.56	5.66	3.63	3.31	8.03	8.81

[1] Diluted flour at 12.0% protein level (13.5% moisture basis).
[2] Glutens prepared from the various wheat varieties and blended with a constant starch substrate at a uniform protein level of 13.5% (13.5% moisture basis).

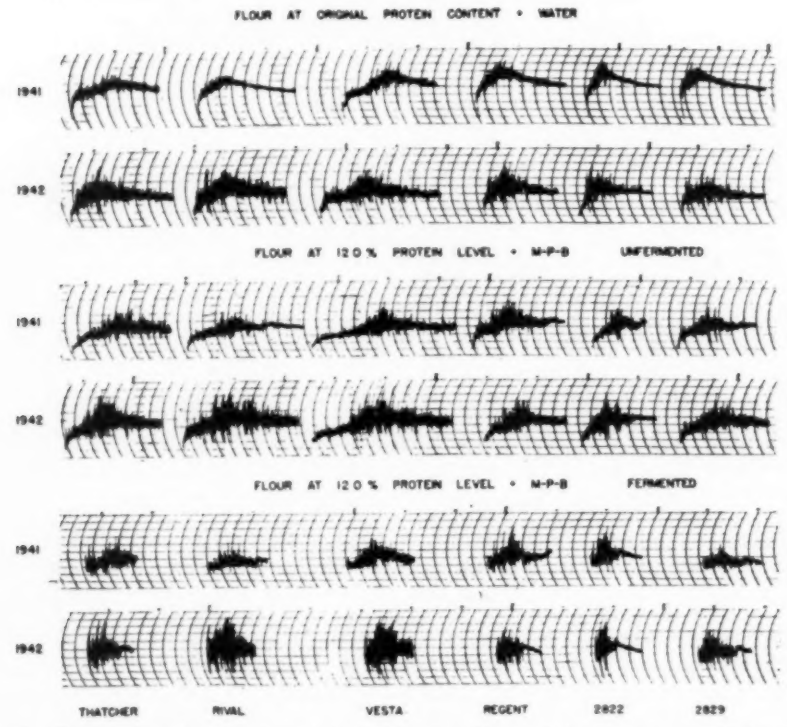

Fig. 2. Comparative mixograms from six hard red spring wheat varieties grown comparably in 1941 and 1942. The lower two lines of curves were obtained by remixing fermented doughs.

development stage and range of stability in both the flour and blend data. These differences seem to be correlated for the corresponding flour and blend mixograms.

Figure 2 shows comparative mixograms obtained from six of the hard red spring wheat varieties grown in 1941 and 1942. Three treatments are represented: flour-water mixes; flour with the malt-phosphate-bromate baking formula unfermented; and flour with the baking formula and 3-hour fermentation. It is apparent that all 1941 curves were

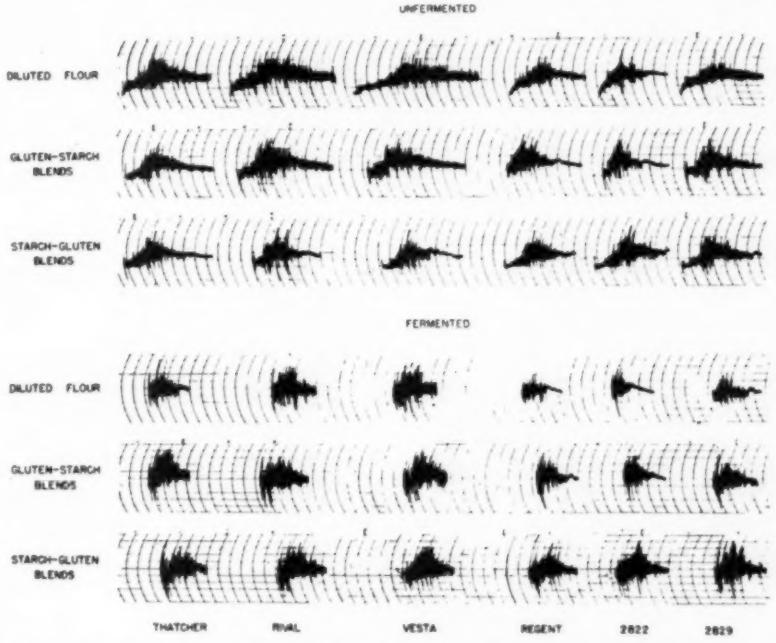

Fig. 3. Mixograms secured from the original flours diluted to 12.0% protein level blended with (a) glutens prepared from the different wheat varieties and a common starch; (b) starches prepared from the wheats and blended with the same hard red spring wheat gluten. Blends made at a protein level of 13.5%.

of a different pattern from the 1942 curves. This difference is very noticeable in the flour-water doughs. Most of the 1941 curves are narrow and rise to a relatively sharp peak. Rival and 2822 are the most noticeable in these respects. The 1942 curves are much broader and less sharply peaked. These characteristic differences are shown by all the methods employed. The baking formula ingredients increase the width of all the curves appreciably. Fermentation, followed by remixing, tended to shorten the curves and reduce the height. Some of the varietal characteristics carry through the fermentation and remixing procedure.

Figure 3 presents a few mixograms taken from comparisons between the original 32 flours and the dried gluten and starch blends.

One set of curves was obtained by mixing the doughs with the malt-phosphate-bromate formula, while the other group was made by remixing after fermentation. Varietal patterns can be discerned in the diluted flour curves and with the blends made with the constant starch substrate and different glutens. Variable starches with the constant gluten, however, do not show these characteristic properties. It is evident that mixing requirements are influenced by the gluten and not by the starch; similar results were obtained by Swanson and Andrews (1943) in studies of hard red winter wheats.

Table III shows an analysis of variance of the data from series one.

TABLE III

ANALYSIS OF VARIANCE OF THE MIXOGRAM DATA FOR EIGHT WHEAT VARIETIES GROWN IN 1942

Source of variation	Degrees of freedom	Variances			
		Dough development stage	Range of dough stability		Curve height
			Total	Post-optimum	
Between varieties	7	20.32 [1]	13.74 [1]	4.67 [1]	0.22 [2]
Between methods	2	98.14 [1]	14.46 [1]	7.89 [1]	11.17 [1]
Between stations	3	3.57 [1]	2.59 [2]	0.72	4.23 [1]
Interactions:					
(Varieties × methods)	14	1.93 [1]	1.02	0.72 [2]	0.13
(Varieties × stations)	21	0.84 [2]	0.42	0.24	0.12
(Stations × methods)	6	1.14 [2]	2.83 [1]	1.57 [1]	0.23
(Varieties × methods × stations)	42	0.38	0.81	0.34	0.08
Total	95				

[1] Denotes significance exceeding 1% point.
[2] Denotes significance exceeding 5% point.

The triple interaction, varieties × methods × stations, has been used as a measure of error. It is apparent that very significant differences exist between wheat varieties in all except one of the measurements; for curve height the difference is significant at the 5% point but not the 1%. Differences in all four properties are extremely significant between methods; Table I shows that this is chiefly because of the effect of fermentation and remixing upon the dough; and this is in accord with *a priori* expectations. The station differences are significant at the 1% point for dough development and curve height, while total range of dough stability is significant at the 5% point only; no significant variations in post-optimum dough stability were found. Significant interactions are shown between varieties and methods for dough development and post-optimum range of stability. For varieties × stations the only significant effect is for dough develop-

ment stage, while for stations × methods all interactions are significant except for curve height.

Table IV, which deals with series two, shows results somewhat similar to those in Table III. One mixing method, using the malt-phosphate-bromate formula, was employed. Varietal differences in

TABLE IV

ANALYSIS OF VARIANCE OF THE MIXOGRAM DATA FOR SIX WHEAT VARIETIES GROWN IN 1941 AND 1942

Source of variation	Degrees of freedom	Variances		
		Dough development stage	Range of dough stability	Curve height
Between varieties	5	40.07 [1]	12.30 [1]	0.15
Between years	1	0.07	0.90	4.75 [1]
Between stations	3	9.29 [1]	1.35	1.19 [1]
Interactions:				
(Varieties × years)	5	0.58	0.90	0.20
(Varieties × stations)	15	1.43	0.43	0.14
(Years × stations)	3	3.92 [2]	1.56 [2]	0.06
(Varieties × years × stations)	15	0.92	0.46	0.16
Total	47			

[1] Denotes significance exceeding 1% point.
[2] Denotes significance exceeding 5% point.

dough development stage and range of stability are very marked, while curve height differences are not significant. Station differences are very significant for dough development and curve height, but not for range of stability, while annual differences are significant only for curve height. For the interactions, varieties × stations are not significant throughout; years × stations are significant for dough development stage, and range of stability at the 5% point, while curve height is not significant; varieties × years interactions are quite insignificant.

The analysis of the gluten-starch blend data for the third series is shown in Table V. The varietal effect on stage of dough development and range of stability is extremely significant, while for curve height there is a significant effect at the 5% point. The effect of method is also very marked for the three properties studied. Station differences are not significant except for curve height which is significant at the 5% point. The interaction of varieties × methods is significant at the 5% point for dough development stage and range of stability, and for varieties × stations only in dough development. There was no significant interaction between stations and methods.

The data for starch-gluten blends showed results of but minor interest. There were, of course, very significant differences between methods. Varietal and station differences were of little importance,

TABLE V

ANALYSIS OF VARIANCE OF THE MIXOGRAM DATA FOR THE GLUTEN BLENDS WITH A COMMON STARCH

Source of variation	Degrees of freedom	Variances		
		Dough development stage	Range of dough stability	Curve height
Between varieties	7	6.47 [1]	7.42 [1]	0.22 [2]
Between methods	1	35.10 [1]	18.71 [1]	0.96 [1]
Between stations	3	0.16	0.53	0.31 [2]
Interactions:				
(Varieties × methods)	7	0.31 [2]	1.11 [2]	0.13
(Varieties × stations)	21	0.31 [2]	0.36	0.12
(Stations × methods)	3	0.08	0.31	0.14
(Varieties × methods × stations)	21	0.11	0.35	0.07
Total	63			

[1] Denotes significance exceeding 1% point.
[2] Denotes significance exceeding 5% point.

TABLE VI

CORRELATION COEFFICIENTS FOR THE MIXOGRAM DATA

	Variables correlated		r_{xy} [1]
	X	*Y*	
Dough development stage	Unfermented doughs, flour 12% protein level, cm	Fermented doughs, flour 12% protein level, cm	+0.740
Range of stability	Unfermented doughs, flour 12% protein level, cm	Fermented doughs, flour 12% protein level, cm	+0.560
Dough development stage	Unfermented doughs, gluten-starch blends, cm	Fermented doughs, gluten-starch blends, cm	+0.878
Range of stability	Unfermented doughs, gluten-starch blends, cm	Fermented doughs, gluten-starch blends, cm	+0.709
Dough development stage, unfermented doughs	Flour 12% protein level, cm	Gluten-starch blends, cm	+0.720
Range of stability, unfermented doughs	Flour 12% protein level, cm	Gluten-starch blends, cm	+0.705
Dough development stage, fermented doughs	Flour 12% protein level, cm	Gluten-starch blends, cm	+0.856
Range of stability, fermented doughs	Flour 12% protein level, cm	Gluten-starch blends, cm	+0.653
Dough development stage, unfermented doughs	Flour 12% protein level, cm	Starch-gluten blends, cm	+0.390
Range of stability, unfermented doughs	Flour 12% protein level, cm	Starch-gluten blends, cm	+0.355
Dough development stage, fermented doughs	Flour 12% protein level, cm	Starch-gluten blends, cm	+0.183
Range of stability, fermented doughs	Flour 12% protein level, cm	Starch-gluten blends, cm	−0.083
Flour, original protein content	Wheat protein content, %	Height of curve, cm	+0.336
Flour, original protein content	Wheat protein content, %	Flour loaf volume, cc	+0.661
Flour doughs, 12% protein level, fermented	Dough development stage, cm	Post-optimum range of stability, cm	+0.794
Flour doughs, 12% protein level, unfermented	Dough development stage, cm	Post-optimum range of stability, cm	+0.698
Flour-water doughs, 12% protein level	Dough development stage, cm	Post-optimum range of stability, cm	+0.471
Gluten-starch blends, unfermented	Dough development stage, cm	Post-optimum range of stability, cm	+0.809
Gluten-starch blends, fermented	Dough development stage, cm	Post-optimum range of stability, cm	+0.596

[1] Value of r_{xy} at 5% point 0.352; at 1% point 0.464.

and it is evident that wheat starch had no important role in determining mixogram pattern in these flours.

The more important correlation coefficients which have been calculated for the relations between many of the variables studied are given in Table VI. For dough development stage and range of stability, there is a positive relation between unfermented and fermented doughs with both flours at 12% protein and gluten-starch blends. There are also significant positive relations between the flour doughs and the gluten-starch doughs in each instance, including both unfermented and fermented doughs. In the comparisons between flour and starch-gluten doughs, however, the relations are of little interest. These findings are further evidence that the curve pattern is determined by the gluten. Wheat protein content apparently had little influence on curve height in this series of samples. A positive relation was found between dough development stage and post-optimum range of stability, which would seem to indicate that flours with long mixing requirements also tend to have greater mixing stability.

The data presented indicate that hard red spring wheat varieties may be differentiated to a considerable extent by their mixogram properties. These properties are also significantly affected by the environmental conditions under which the wheats are grown and are inherent in the gluten component. It seems probable that dough mixing requirements are positively correlated with dough stability during mixing.

Summary

The mixogram properties of a number of hard red spring wheat varieties were examined in respect to (1) the significance of varietal and environmental differences, and (2) the comparative influence of the gluten and starch component of wheat on these differences. Three mixing procedures were also compared respecting their effects on these properties.

Dough development stage, range of dough stability, and curve height were determined by simple measurements taken from the mixogram curves. Significant differences were found among varieties and stations for these properties among eight varieties grown at four locations in 1942. There were highly significant differences between mixing methods. For six varieties grown at four stations for two years, significant differences between years were found only for curve height. In this series, very significant differences were found between varieties for dough development stage and range of stability. Varietal mixing characteristics were reasonably consistent for the two crop

years. In both series, station differences were highly significant for dough development stage and curve height.

Mixograms obtained from blends of gluten and starch showed quite conclusively that varietal differentiation in mixing properties is related to the gluten and not to the starch component.

Fermentation did not change the relations between corresponding mixogram properties. Doughs having a long dough development stage tended to have a greater range of stability.

Several of the wheats examined differed significantly in curve properties from varieties that have been accepted as satisfactory by the milling industry.

The value of the mixograph as an accessory tool in the evaluation of the quality of hard red spring wheat varieties has been demonstrated by the work described.

Acknowledgment

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THE USE OF THE MIXOGRAM IN EVALUATING QUALITY IN SOFT WHEAT VARIETIES [1]

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New varieties and selections of soft wheat developed by plant breeders are commonly evaluated by comparing their quality characteristics with those of older varieties whose commercial utility is known. Undoubtedly the best criteria of quality in wheat flours are performance tests in which the flours are actually baked into the products for which they are intended. Unfortunately, evaluation of soft wheat is complicated by the multiplicity of products into which the flours are processed and the lack of standardized baking tests which might serve as adequate indices of quality. For the characterization of soft wheat breeders' samples therefore, it has been necessary to rely largely on chemical, physical, and physiochemical tests. The apparent viscosity of an acidulated flour-water suspension is an example of the type of test which has proved valuable as a quality index.

A recent physical test for wheat flour doughs which has proved valuable for the characterization of wheat varieties, especially in the bread-production field, is the recording dough mixer or mixograph. The instrument, developed by Swanson and Working (1933), was "designed to measure and record automatically the rate of dough development, the duration of resistance against mechanical action, and the rate and extent of increase in mobility of dough as a result of mechanical action." Since these characteristics of dough are a result of inherited traits of wheat varieties, some investigators (Swanson and Working, 1933; Swanson, 1936 and 1939) regard the mixograph as particularly valuable in wheat improvement work in differentiating characteristics due to inheritance. In a study of tests applied to hard red winter flours, Larmour, Working, and Ofelt (1939) concluded that where the protein content is known, the mixograms are most useful in establishing qualitative differences between varieties that may or may not be equal in baking strength.

In connection with a study of baking tests applied to 10 soft wheat varieties, Shellenberger, Hodler, and Nelson (1942) pointed out that mixograms were useful also for indicating differences in the dough characteristics of soft wheat flours.

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[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Ohio Agricultural Experiment Station.

[2] Chemist, Assistant Technologist, and Agent, respectively, Federal Soft Wheat Laboratory, Wooster, Ohio. C. E. Bode and H. K. Heizer are now on military furlough with the U. S. Army.

The purpose of the work reported in the present paper was to investigate the mixogram as a measure of quality in the characterization and evaluation of the soft wheat variety samples received at the Federal Soft Wheat Laboratory.

Material and Method

A National Swanson-Working mixograph, operated in an air-conditioned laboratory maintained at 80 ± 1.0°F, was used in this study.

The samples of wheat were milled on an Allis-Chalmers experimental mill. The patent, consisting of break flours and first two reductions, was kept separate from the last two or three reductions until ash determinations could be made on these streams. Enough of

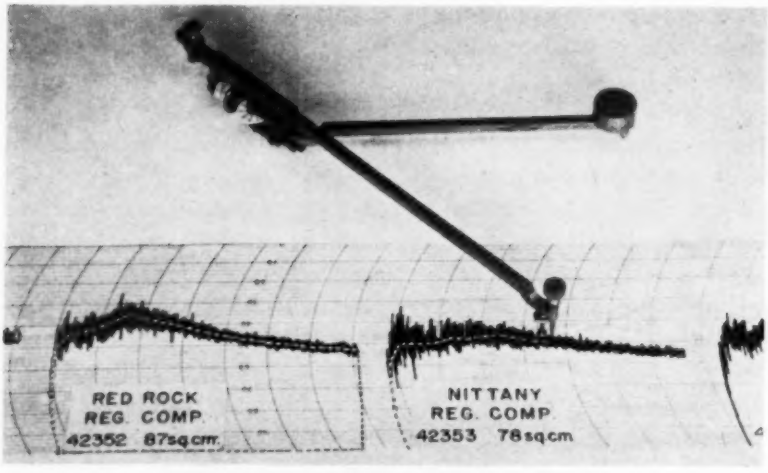

Fig. 1. Measuring mixogram areas with planimeter.

the latter streams was then added to bring the ash content of the flour used for quality tests to about 0.42%. It has been found that on the average this amounts to about 95% of the straight flour.

In developing a procedure suitable for routine samples, two objectives were considered: (1) coverage of the entire range in strength normally found in wheats in the eastern soft wheat region without adjustment of the mixograph, and (2) a single-figure score for expressing general strength or characteristics.

A procedure has been developed which seems to incorporate these features. This procedure includes the use of 35 g of flour (13.5% moisture basis), a spring tension of 8, a mixer head speed of 90 rpm, and a constant absorption of 54%.[3]

[3] This absorption represents an average value for soft wheat flour as determined for bread baking purposes.

In attempting to solve the problem of a single-figure score for expressing the general characteristics of a mixogram, various measurements, including some of those suggested by Johnson and Swanson (1942), were made on a large number of mixograms. The measurement which seemed best to express the gluten strength of each flour sample is the "area under the mixogram."

The measurements reported in this study were made with the Keuffel and Esser Company planimeter shown in Figure 1. The dotted lines in this figure represent the path followed by the planimeter pointer in outlining the "area under the mixogram." The results are read from the planimeter scale in square centimeters. A uniform lapsed time of exactly 7 minutes from start of mixing was included in each area. For simplification, the measurement is referred to in this paper as "mixogram area."

It has been found that although the general characteristics of a mixogram are readily reproducible, there is often a substantial difference in area between replicates even under carefully controlled conditions. On the basis of 1941 and 1942 results, the standard error of a single result has been determined to be 1.745 sq cm. Thus a difference of at least 3.5 sq cm is required for significance between means of duplicates, and 5.0 sq cm for a single mixogram on each sample.

Results

Characterization of Varieties by Mixograms. The use of mixograms in the characterization and evaluation of soft wheat varieties is illustrated by results obtained with the uniform soft winter wheat nursery samples. This series is grown at a number of state experiment stations each year for observations relative to winter hardiness, yield, disease reaction, etc., and includes a number of the most promising newer varieties or selections from the soft wheat region, in addition to many of the older varieties whose commercial utility is known. The results considered in this study involve 29 varieties grown in 1941 at four locations in Ohio (Holgate, Wooster, Columbus, and Miami) and one each in New York, Illinois, Michigan, Indiana, and Kentucky. The grain produced at these locations was composited by variety previous to milling. This series of samples is considered especially suitable for comparing methods of evaluating inherited qualitative differences in gluten characteristics, since differences in protein quantity among these samples are relatively small, and those which do occur are either random differences or are due to an inherited tendency of certain varieties for high or low protein content.

The older varieties which are included to serve as standards of comparison were chosen because they represent the range in gluten

strength from hard red winter to soft white wheats. The ability of the mixograph to differentiate varieties over this range is demonstrated in the mixograms, shown in Table I and Figure 2. Arrangement of

TABLE I

MIXOGRAM AREAS OF VARIETIES GROWN IN EASTERN UNIFORM SOFT WINTER WHEAT NURSERIES; VARIETY SAMPLES COMPOSITED FROM GRAIN GROWN AT NINE LOCATIONS IN 1941

Variety	Mixogram area	Variety	Mixogram area	Variety	Mixogram area
	sq cm		*sq cm*		*sq cm*
Kharkof	94	Clarkan	85	Fultz Sel. Hung.	76
Purkof	92	Baldrock	84	Fairfield	74
Brill	92	Fulcaster	83	Gladden	72
		Minhardi	82	Early Premium	71
Mich. Amber	91	Wisc. Ped. No. 2	82	Purdue No. 7	71
Minturki	91	Prairie	80		
Forward	88	Trumbull	80	Yorkwin	67
Red Rock	88	Purdue No. 1	80	Wabash	67
Kawvale	86	Thorne	80	Am. Banner	62
		Nittany	80	Junior No. 6	57
		Purplestraw	79		
		Illinois No. 2	78		

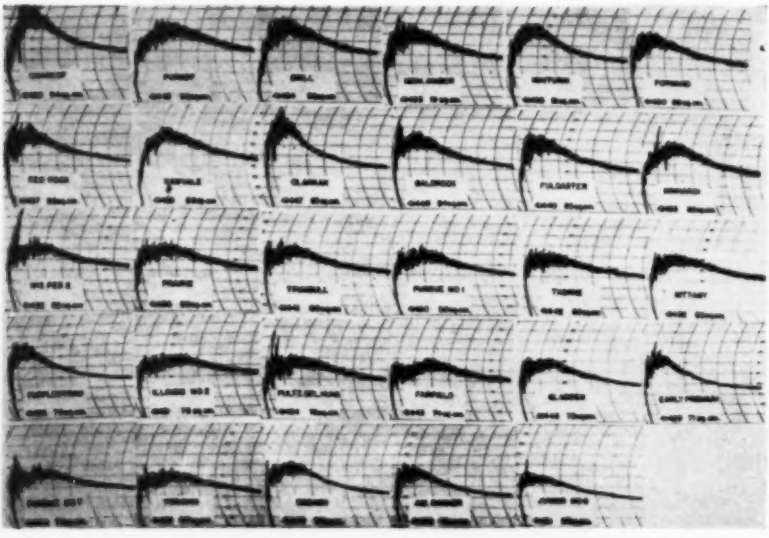

Fig. 2. Mixograms of varieties grown in eastern uniform soft winter wheat nurseries; samples composited from grain grown at nine locations in 1941.

the varieties according to mixogram area is consistent with what is known regarding the relative quality characteristics of the varieties as shown by various physicochemical and baking tests conducted over a period of years.

Of the three varieties having the greater mixogram areas, Kharkof, Brill, and Purkof, the first two are hard red winter varieties, and one parent of Purkof was a hard red winter wheat. The mixograph thus confirms the results of several years' experience with other quality tests in characterizing these three varieties as outstanding among the varieties in this series in gluten strength. Satisfactory commercial utilization of these varieties is limited to bread production.

Ranking the varieties according to mixogram area places in the next group Michigan Amber, Minturki, Forward, Red Rock, and Kawvale. The first mentioned is a selection from a cross between a hard red winter (Turkey) and a soft wheat (Odessa). Other years' results with Minhardi suggest that it also belongs in this group, although the mixogram area of this sample did not so place it. These varieties have been characterized by other quality tests as soft red winter wheats with gluten strength somewhat weaker than the first group, suitable for the most part for family trade flours.

According to mixogram area, Clarkan, Baldrock, Fulcaster, Wisconsin Pedigree No. 2, Prairie, Trumbull, Purdue No. 1, Thorne, Nittany, Purplestraw, and Illinois No. 2 might be included in the next group. Although there is some range in quality exhibited among members of this group, the older varieties are known to produce satisfactory soft wheat flours for such purposes as cakes, cracker sponges, etc. The results of laboratory quality tests agree in characterizing these varieties as medium strength soft red wheats.

From the standpoint of general quality tests there is no clear line of demarcation between the varieties listed in the preceding paragraph and the following: Fultz Sel. × Hungarian, Fairfield, Gladden, Early Premium, and Purdue No. 7. Of this group, Fultz Sel. × Hungarian, Gladden, and Purdue No. 7 in particular have shown a slight but distinct tendency toward a softer gluten than the other members of the two groups. In this respect the mixogram area and other quality tests are apparently in agreement.

The varieties placed in the weakest group according to mixogram area are, with the exception of Wabash, soft white wheats. Physicochemical and baking tests agree in characterizing Wabash and Yorkwin as being of about equal gluten strength; American Banner and Junior No. 6 as being distinctly softer in quality.

Although in general the varieties are thus arranged in logical groups according to quality reputation and mixogram areas, it should perhaps be pointed out that the smallest area of a variety in one group may differ very little from the largest area of a variety in the following group.

Correlation Coefficients between Mixogram Areas and Results of Other Quality Tests. Additional evidence of the general agreement between mixogram areas and the results of other tests used for quality characterization in this series is afforded by the simple correlation coefficients presented in Table II.

TABLE II

CORRELATION COEFFICIENTS BETWEEN MIXOGRAM AREA, FLOUR PROTEIN, DOUGHBALL TIME, VISCOSITY VALUES, LOAF VOLUME, AND COOKIE SPREAD FACTOR FOR 29 VARIETIES GROWN IN EASTERN UNIFORM SOFT WHEAT NURSERIES IN 1941

(Value required for significance at 1% point for 29 varieties $r = \pm 0.470$)

	Flour protein	Doughball time	Viscosity		Loaf volume M-P-B [1]	Cookie spread factor
			20-g flour	2-g protein		
Mixogram area	+0.659	+0.674	+0.821	+0.693	+0.717	−0.810
Flour protein		+0.379	+0.688	+0.318	+0.642	−0.595
Doughball time			+0.576	+0.583	+0.488	−0.529
Viscosity, 20-g flour				+0.839	+0.736	−0.768
Viscosity, 2-g protein					+0.573	−0.653
Loaf volume, M-P-B[1]						−0.696

[1] Formula B, Bayfield (1941).

Mixogram area gave a high positive correlation with 20-g flour viscosity ($r = +0.82$) and an equally high negative correlation ($r = -0.81$) with cookie spread factor (Hanson, 1943). Mixogram area was correlated with loaf volume to a somewhat lesser degree ($r = +0.72$) although the relationship was highly significant as was true also of the coefficients between mixogram area and 2-g protein viscosity ($r = +0.69$) and doughball time ($r = +0.67$).

The 20-g flour viscosity determination was, in general, correlated with the other quality tests to about the same degree as was mixogram area. Cookie spread factor and loaf volume were correlated less highly with the results of other tests than was either 20-g flour viscosity or mixogram area.

Influence of Environment on Quality. The modifying effects of environmental factors on the quality characteristics of wheat varieties are well recognized. It has been shown by Swanson (1939) that environmental factors, particularly as they influence protein content, cause marked variations in the mixograms of a wheat variety. The extent to which mixograms of variety samples grown in the eastern soft wheat region are influenced by environmental factors can be illustrated by mixograms of five well-known commercial varieties grown at four locations in 1941, shown in Figure 3.

These five varieties represent the range of quality found in the eastern area. Purkof is a semi-hard to hard red winter wheat and is too strong for most purposes except bread. Red Rock when grown under most environmental conditions is regarded as too strong for a

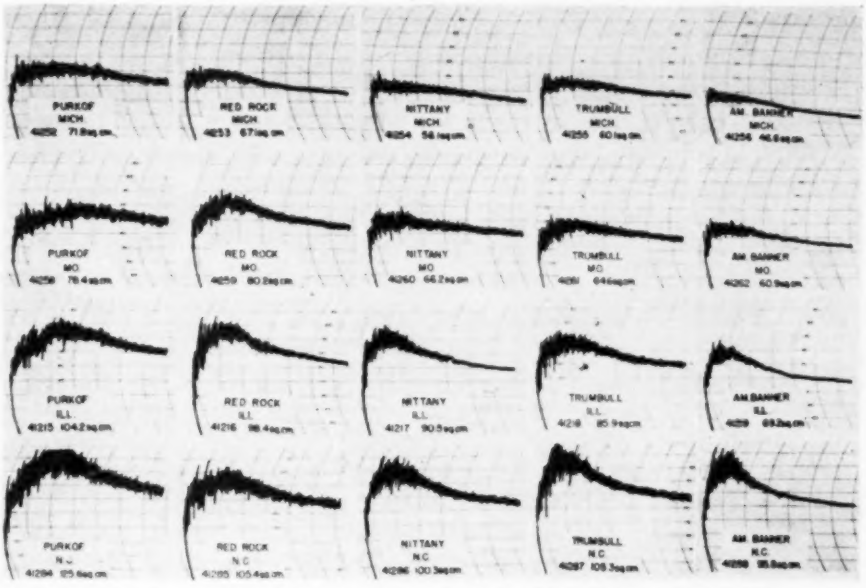

Fig. 3. Effect of environmental factors on mixogram characteristics of five standard varieties grown at four stations in 1941.

satisfactory pastry flour. Nittany and Trumbull are soft red winter wheats of recognized pastry quality. American Banner is a white wheat satisfactory for weaker pastry purposes.

The protein content of the varieties at each station was much the same, although some variation may be noted. The protein contents together with the mixogram areas are given in Table III.

TABLE III

PROTEIN CONTENTS AND MIXOGRAM AREAS OF FIVE WHEAT VARIETIES GROWN AT FOUR LOCATIONS IN THE EASTERN SOFT WHEAT REGION IN 1941

Location	Purkof		Red Rock		Nittany		Trumbull		American Banner		Average	Average
	Protein	Mixogram area	Protein	Mixogram area	Protein	Mixogram area	Protein	Mixogram area	Protein	Mixogram area	Protein	Mixogram area
	%	*sq cm*	%	*sq cm*	%	*sq cm*	%	*sq cm*	%	*sq cm*	%	*sq cm*
East Lansing, Mich.	7.2	72	7.3	67	7.3	56	7.8	60	7.0	47	7.3	62.0
Columbia, Mo.	8.1	78	9.0	80	8.9	66	9.0	65	8.6	61	8.7	70.0
Urbana, Ill.	10.5	104	11.7	98	11.1	91	11.1	86	10.1	69	10.9	89.6
Statesville, N. Car.	14.5	126	13.0	105	13.2	100	13.6	105	12.7	96	13.4	106.4

The immediate attainment of minimum mobility of the dough, characteristic of low-protein soft wheat flours, is well illustrated by the mixograms of the Michigan samples. Only the stronger gluten varieties, Purkof and Red Rock, show some development of gluten with increased mixing time.

When the wheat was grown under environmental conditions (Missouri) resulting in an average flour protein content of 8.7%, the mixograms of even the softer varieties (Nittany, Trumbull, American Banner) began to demonstrate some dough development with mixing. With the samples grown under Illinois conditions, which contained 2.2% more protein on the average than the Missouri samples, there was a further marked increase in mixing time to the point of minimum mobility. These mixograms also show, however, the characteristic rapid narrowing in the weakening slope noticeable in the curves from the lower protein samples.

The mixograms from the North Carolina samples, which contained an average of 13.4% protein, are characterized by no further increase in mixing time to the point of minimum mobility, but by some increase in general width of curve, especially at that point. This level of protein is seldom found in the eastern soft wheat region and represents an average increase of 2.5% over the Illinois samples.

These mixograms demonstrate the effect environment may have on the mixogram characteristics of a variety and serve to emphasize the necessity in comparative evaluation of varieties of using samples grown under uniform environment and with the same relative protein content. It can be noted, however, that at any one location, the five varieties are very well differentiated except for Nittany and Trumbull, which are known to be similar in quality. Although in several instances the differences in mixogram areas do not reach the 5 sq cm required for statistical significance, other characteristics of the mixograms do indicate definite differences in gluten quality. The Purkof and Red Rock samples from Michigan and Missouri are examples of such samples.

Summary

A satisfactory procedure is described for obtaining mixograms from soft wheat flours for characterizing variety samples over the entire range in flour strength found among samples grown in the eastern soft wheat area.

A single-figure score obtained by measuring the area under the flour mixogram for a mixing time of 7 minutes is suggested as a general expression of strength due to gluten quality and quantity.

In a test of 29 varieties representing a wide range in quality, ranking according to mixogram area was consistent with what is

known regarding the relative quality of the varieties. High correlation coefficients were obtained between mixogram areas and the results of other quality tests, such as 20-g flour viscosity and cookie and bread baking.

Since varietal quality characteristics as expressed in mixograms are modified greatly by environment, comparative evaluation of soft wheat varieties by mixograms can be made only on samples grown under uniform conditions.

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SIFTED WHEAT MEAL MIXOGRAMS FOR SELECTING SOFT WHEAT VARIETIES

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(Read at the Annual Meeting, May 1943; received for publication July 28, 1943)

Quality evaluation of soft wheat samples offers serious difficulties. The flour is used for diverse purposes, and specifications often range within narrow limits, necessitating sensitive tests. Methods have not been developed to the place where they can be interpreted readily in terms of flour usefulness. With new strains, especially in the earlier stages of a breeding and testing program, even greater difficulty has been encountered.

Mixograms seemed to offer possibilities, if modifications of technique could be made. For use with nursery samples, small amounts of grain must suffice, and labor be reduced to a minimum. Milling is almost out of the question. Tests on ground wheat meal appeared to

be the most hopeful. Malloch (1938) used wheat meals in a machine developed by him, and obtained significant correlation ($r = +0.88$) between the breaking points of meal and flour doughs. More recently Johnson and Swanson (1942) have published a paper dealing with hard wheat meal mixograms. They were able to obtain satisfactory curves, closely similar to the flour curves in their essential characteristics.

The present study was undertaken to explore the possibilities of wheat meal mixograms in the evaluation of soft winter wheat nursery material.

Experimental

Development of Technique. In the spring of 1940, samples of several varieties known to differ in quality characteristics were ground in a Wiley mill, and also in a Jacobson hammer mill and sifted through a 10xx bolting silk. These meals were taken to the Mennel Milling Company Laboratory at Fostoria, Ohio, where mixograms were made using their Swanson-Working recording dough mixer or mixograph. Results were so promising that it was decided to investigate the possibilities further.

A mixograph was purchased by the Ohio station, and exploratory experiments begun on the 1940 crop. Samples were ground in the Wiley mill, Jacobson hammer mill, Labconco burr grinder, Allis-Chalmers experimental mill, and Bantam mikro pulverizer, using various screens and adjustments. The ground wheat was sifted through 64 GG, 6xx, 10xx, and 14xx bolting silks. The mikro pulverizer was considered the most satisfactory grinding device when used with the 0.035-inch herringbone screen. The 10xx bolting silk was selected for sifting.

Sifted meal prepared in this way showed finer particle size distribution than flour from the Allis experimental mill. Some high-ash material was removed in the coarse fractions, but using silks finer than 6xx did not reduce ash appreciably. Protein in the meal also dropped below the wheat samples, as would be expected, and was lowest where the "throughs" were least.

In the fall of 1940, mixograms were made using a head speed of 60 rpm, and a tension setting of 6. This procedure gave poor results; the curves were flat in outline with indistinct maxima in most cases. Where there was much irregularity in the first part of the curve, the true course was often seriously masked. A head speed of 90 rpm was adopted in 1941, with a tension setting of 8, and greatly improved results were obtained.

In general, from the 1940 work it was noted that the finer the meal, the more uniform and clear was the course of the curve; that the silk used to sift the meal was much more important than the screen used in

the micro pulverizer; that low-moisture wheat resulted in high ash and poorer curves; that a 10xx silk was sufficiently fine to separate the meal.

In the fall of 1941, further exploratory tests were made. Wheat was tempered to various levels, and in some cases an additional 2% moisture was added shortly before grinding. The object was to toughen the bran if possible, and reduce the ash in the sifted meal. The attempt was not successful. There appeared to be little advantage in tempering unless samples were below approximately 13% moisture. There was considerable drying of the samples in the mikro pulverizer since it is a very high-speed hammer-type mill with considerable air draft. Accordingly, it was decided to temper to a moisture content of approximately 16% at least 24 hours before grinding.

Violent fluctuations in the first part of the curves were most common on coarse meals, and it was suspected that the hydration of larger particles was slower and less uniform than that of the small particles. Drier meals also gave more trouble, taking longer to settle down to a smooth curve. This result suggested that it might be well to have the meals at as high a moisture as feasible before making the test. Several samples were spread out in a thin layer overnight in a proofing cabinet at high humidity. They took up considerable moisture, but there was no improvement in the curves. Fineness of meal appeared to be the answer to this problem.

The final procedure adopted was as follows: Temper 125 g of wheat to approximately 16% for 24 hours; grind in a Bantam mikro pulverizer, using 0.035-inch herringbone screen; sift 1 minute using No. 120 wire screen (or 10xx silk) and save the "throughs"; determine moisture (5-g samples 30 minutes at 140°C); keep samples at 80°F overnight; weigh out enough meal to contain 30.275 g dry matter (35 g meal at 13.5% moisture); add distilled water (at 80°F) to bring total weight to 55 g and make mixogram immediately; mixograph run 6–7 minutes.

All the work has been carried out in a constant-temperature laboratory maintained at 80°F. Although specific experiments have not been carried out, it has been evident that temperature is critical, and must be maintained within rather narrow limits for satisfactory results.

Agreement with Flour Mixogram. Concurrently with this work, Morris, Bode, and Heizer (1944) were developing a technique for use with soft wheat flours. They have found the test one of the best for evaluating such samples. It appeared, therefore, that if sifted meal mixograms agree well with those on flour, the value of the test could be estimated with considerable confidence. To this end, tests were made for two seasons, using the composite uniform soft wheat nursery samples for 1940 and 1941. These included 30 varieties the first year and 29 the second.

Several methods of comparison were used. Figure 1 presents flour and meal mixograms for 10 varieties for the two seasons. Agreement between the two sets is striking, and was equally good for the remaining varieties.

Areas were determined on flour mixograms for 7, 6, 5, and 4 minutes, and on the meal mixograms for 6, 5, and 4 minutes, employing the method described by Morris, Bode, and Heizer (1944). These data were correlated with corresponding data for 20-g flour viscosity. Results are given in Table I.

TABLE I

CORRELATION BETWEEN 20-g FLOUR VISCOSITY AND MIXOGRAM AREA
(Mixogram areas for several time intervals)
(1% level for r—1940 = + 0.463, for 1941 = + 0.470)

	1940 (30 samples)			1941 (29 samples)		
	7 min	6 min	5 min	7 min	6 min	5 min
Flour	+0.860	+0.843	+0.818	+0.821	+0.810	+0.817
Meal	——	+0.884	+0.858	——	+0.806	+0.794

There is no indication here that mixogram areas are not as satisfactory a measure of quality in meal as in flour.

Correlations for the 7- and 6-minute flour mixogram areas with the 6-minute meal mixogram areas were + 0.923 and + 0.908 respectively for the 1940 samples; and + 0.927 and + 0.919 for the 1941 samples. These are highly satisfactory values.

TABLE II

MINIMUM, MAXIMUM, AND MEAN MIXOGRAM AREAS ON FLOUR AND MEAL 1940 AND 1941

	Flour mixograms				Meal mixograms	
	7 min		6 min		6 min	
	1940	1941	1940	1941	1940	1941
	sq cm	*sq cm*	*sq cm*	*sq cm*	*sq cm*	*sq cm*
Minimum	56.8	57.1	53.2	51.9	47.8	48.3
Maximum	102.6	93.8	92.0	82.3	76.4	87.1
Mean	82.6	79.7	75.1	70.5	65.0	67.8

Mean, maximum, and minimum mixogram areas for flour and for meal are presented in Table II. Almost without exception, sifted meal mixograms gave smaller areas than flour mixograms. The range of area values within a series was variable, but was somewhat less for meal mixograms as a rule.

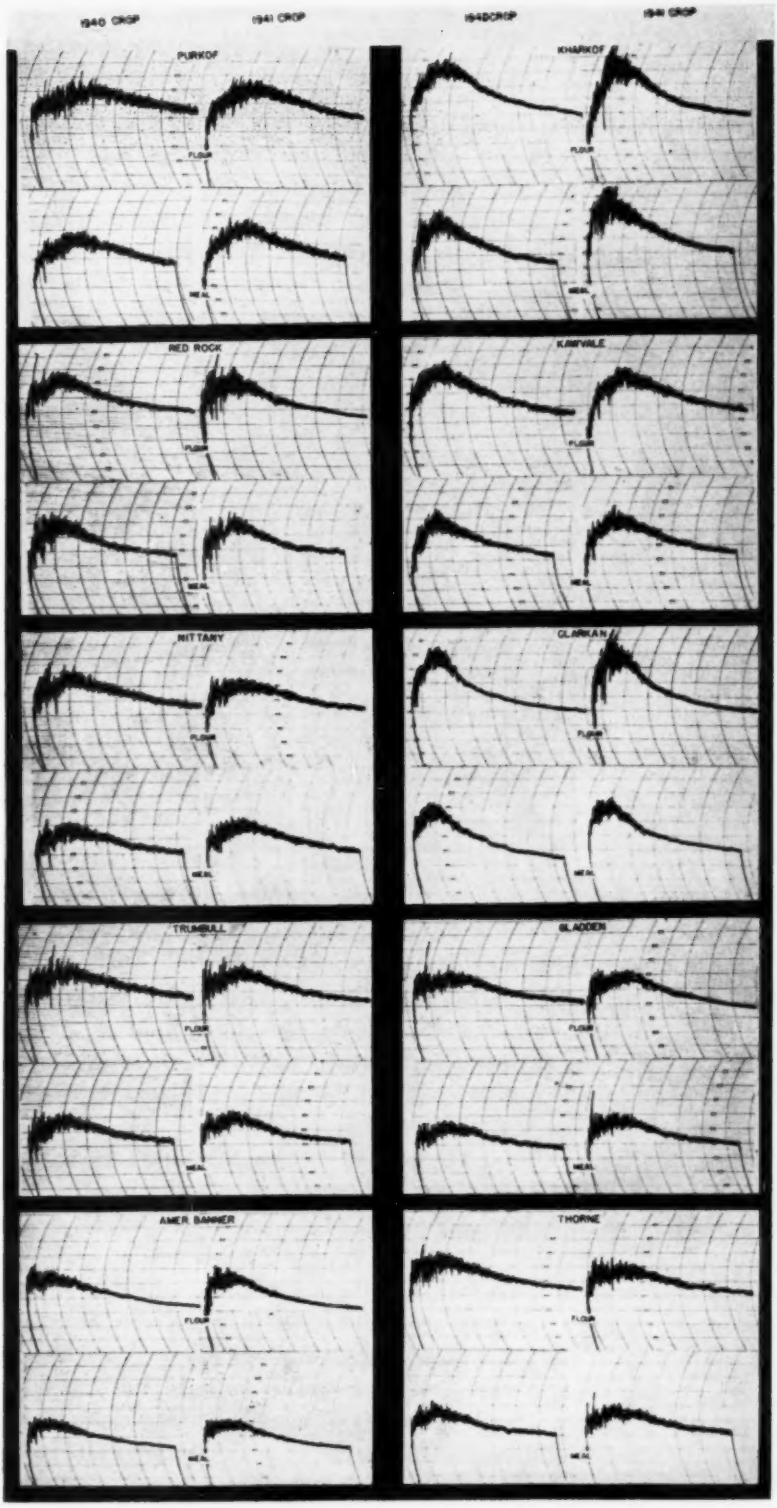

Fig. 1. Flour and meal mixograms from uniform nursery composites 1940 and 1941.

Value with Wheat Nursery Material. It is clear that sifted meal mixograms give almost the same picture as flour mixograms. In the opinion of the writer, the sifted meal mixogram gives more information than any other one test that has been used for nursery material. It has been tried now on three crops grown at five locations with satisfactory results. However, mixograms are considerably affected by protein content and probably to some extent by growing conditions, and reference varieties should be included in every test.

Figure 2 shows mixograms from six varieties at five locations in 1941 and 1942. The curve areas are presented in Table III.

TABLE III

MEAL MIXOGRAM AREAS FOR 1941 AND 1942 NURSERY SAMPLES

Variety	Wooster		S.E. Test		S.W. Test		Holgate	Columbus
	1941	1942	1941	1942	1941	1942	1941	1942
	sq cm	*sq cm*	*sq cm*	*sq cm*	*sq cm*	*sq cm*	*sq cm*	*sq cm*
Trumbull	65.9	60.3	81.4	62.4	83.4	79.5	57.9	71.0
Thorne	65.4	69.3	75.6	56.2	71.1	65.5	58.7	74.6
Gladden	52.9	62.9	65.6	52.1	74.9	64.6	49.1	63.1
T.N. 1153	85.8	—	93.9	67.0	96.7	92.2	74.2	90.9
T.N. 1159	56.4	67.8	73.4	59.9	78.0	65.2	60.3	68.6
H.S. 269	69.1	—	85.6	69.7	85.3	80.8	62.5	82.6
Average	65.9	—	79.3	61.2	81.6	74.6	60.5	75.1

Single mixograms only were made and error could not be estimated. With flour curves, Morris *et al* (1944) have shown that about 5 sq cm difference was required for significance, and it was not likely less with sifted meal. It can be concluded that for 1941 at Wooster, the Southeast test farm, and Holgate, and for 1942 at Columbus, the ranking of varieties was essentially the same. At the Southwest farm in 1941, agreement was not so good, and all the 1942 data, except those from Columbus, were somewhat erratic.

Lines as strong as T.N. 1153 or H.S. 269 can apparently be picked out in any test. All quality tests had proved them to be unsatisfactory. T.N. 1159, a sister selection of T.N. 1153, was acceptable, but not outstanding.

The 1942 crop in Ohio was definitely abnormal. Leaf rust took a severe toll in some areas, and scab and other diseases were also prevalent. The result was low yields and shrivelled grain. Varieties were not all equally affected, nor were the same varieties most seriously injured at all places. Such a crop is obviously unsatisfactory to use for quality studies and accounts for the results obtained. They are

included here to show that mixograms are no more adversely affected than other tests.

A study of results has shown a correlation ranging around + 0.80 between wheat protein and sifted meal mixogram area. Protein is clearly a factor. It is the principal disturbing element in comparing results from two tests. It is sufficiently important that comparison

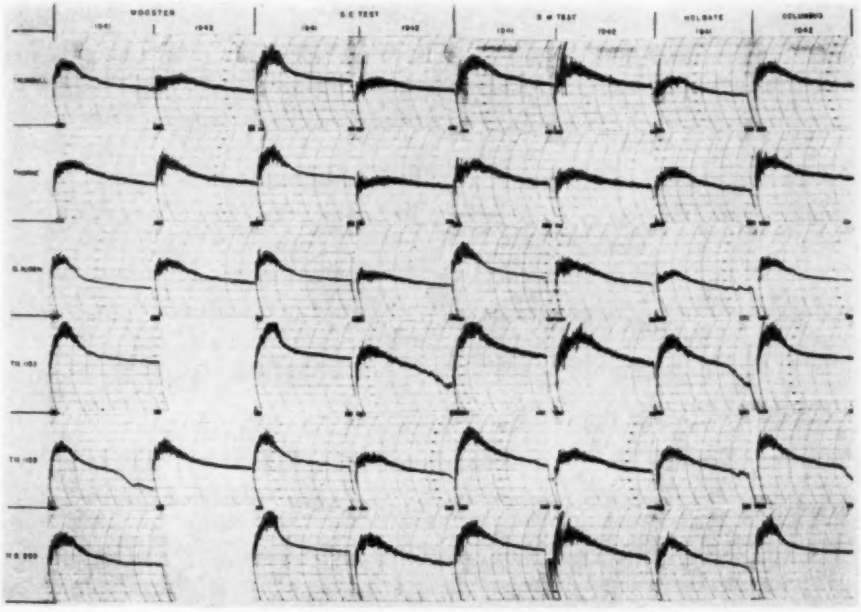

Fig. 2. Meal mixograms for 1941 and 1942 nursery samples.

between strains cannot safely be made unless strains are grown in the same test. On the other hand, when comparing tests, rank of varieties is not seriously affected by high protein at some location or in some season. Experience with the uniform nursery indicates that composites are satisfactory if carefully made.

Johnson and Swanson (1942) noted that the form of mixogram is affected by protein content. Low-protein samples give flatter curves and are more difficult to interpret. The technique outlined has given mixograms which have yielded very useful information in evaluating lines in nursery tests.

Notes on the Test. Although characteristics other than area have been studied, none shows as clear-cut association with baking behavior.

The test does not require an excessive amount of labor. Two men can grind and sift 100 samples per day; one operator using two mixing bowls can make seven mixograms per hour. Including one moisture

determination the total labor need not exceed 30 minutes per sample.

The test appears well adapted for use by plant breeders or by mill or elevator operators wishing a reasonably precise estimate of the quality of a lot of wheat.

Summary

Techniques have been developed for preparing sifted wheat meal from soft wheats, and for making sifted wheat meal mixograms. The meal must be finely ground and the temperature at which the mixograms are made must be carefully controlled. Wheat was tempered to approximately 16% before grinding. Results reported include data from the Federal uniform soft wheat nursery composites (30 varieties, 2 seasons) and from Ohio wheat breeding nurseries (25 strains, 5 locations, 2 seasons).

The area under the wheat meal mixogram curve (6 minutes), adopted as the quality measure, is highly correlated with flour mixogram area and with other quality tests, such as 20-g flour viscosity.

The test is simple and rapid and can be satisfactorily carried out with 125 g of wheat.

Acknowledgment

Wheat samples and flour mixograms from the composite uniform nursery were supplied through the courtesy and cooperation of the Federal Soft Wheat Laboratory at Wooster, an agency of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

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FLOUR BLENDS AND THE QUESTION OF COMPLEMENTARY EFFECTS [1]

KARL F. FINNEY and MARK A. BARMORE [2]

(Presented at the Annual Meeting, May 1941; manuscript received for publication July 1, 1943)

Since an important use of high-protein, good-quality hard red winter wheat flour is for blending with low-protein or poor-quality flours (or a combination of both) to give mixes suitable for bread making, a pertinent question is whether varieties of the former class are accurately evaluated for blending when the flour of each is baked separately.

Flours of certain wheat varieties that appear to be equal in bread-making capacity when baked alone are characterized by considerably different dough-mixing and handling properties. For example, in this laboratory Kharkof and Tenmarq have been shown to be substantially equal in bread-making capacity. On the other hand, curve characteristics as determined by the mixograph suggest that Kharkof is a weaker flour than Tenmarq because of its relatively short developing time and sharp peak. These differences in physical properties suggest that there may be differences in ability to carry low-protein and poor-quality flours even though the baking test indicates equal protein quality when the flours are baked alone. In addition unpublished opinions and studies described in the literature indicate in many cases the presence of complementary effects when flours representing different varieties or classes of wheat are blended in various proportions. In such cases, baking the component flours of a blend separately would not give a reliable measure of the carrying capacity of each.

Harcourt and Purdy (1922) used a lean formula in baking blends of various proportions of Manitoba hard and Ontario soft wheat flours. Their data are reproduced in Figure 1. The solid line is drawn to fit the experimental data, while the broken line represents the theoretical loaf volumes to be expected on the supposition that they are directly proportional to the relative amounts of the flours included in the blend. The data are not in accord with this supposition since those blends containing less than about 40% of Manitoba flour produced smaller loaves than would be expected, while those with 40% or more produced larger loaves than would be expected if there were no "complementary or supplementary effects."

[1] The studies herein reported are a part of the cooperative work carried on by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Agricultural Experiment Stations of the Great Plains Region. Published as contribution number 99 of the Department of Milling Industry, Kansas Agricultural Experiment Station.

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Chapman (1932) states that a 50–50 blend of high-protein hard red spring wheat flour from the Northern Great Plains and a low-protein hard red winter wheat flour from the Southern Great Plains, both of the 1931 crop, was equal in absorption, but had a better color and produced bread with better volume and better texture than that from the hard red spring wheat alone.

Larmour (1931) in studying the relation of protein content and baking quality of hard red spring wheat by means of blends with a club wheat flour increased the proportion of the latter from 40 to 50% be-

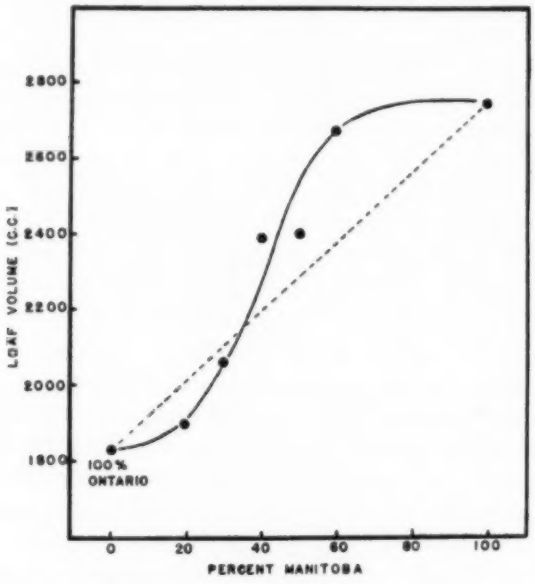

Fig. 1. Loaf volumes for a Manitoba hard, an Ontario soft wheat flour, and their blends (from Harcourt and Purdy, 1922).

cause "in preliminary trials it was observed that some of the higher protein flours showed very little effect of the admixture and it was considered advisable to increase the amount of soft flour sufficiently to insure that even the very strongest flours would show a reduction of volume when compared with the volumes by the bromate formula."

Merritt and Geddes (1943) have reported results of blending experiments showing complementary effects of one flour on another. This, they believe, is due to "the properties of the wheats or flours under study and the baking methods employed."

All of these studies, it will be noted, indicate or imply a complementary effect of one flour on the other. The purpose of this paper is to present certain data bearing on this question.

Complementary is defined according to the dictionary as "serving to fill out and complete, or mutually supplying each other's lack." In the field of cereal chemistry this term has been used in this sense to indicate effects different from the expected average of the components. Flours showing complementary effects in a blend would yield in that blend baking results different than the proportionate average of those produced by the components when baked individually.

Materials and Methods

In the studies reported herein, the flour from each of five varieties of hard red winter wheats, Kharkof, Cheyenne, Nebred, Oro × Tenmarq C.I.[3] 11672, Chiefkan, and one variety of spring wheat, Thatcher, was blended with each of three other flours in 25–75, 50–50, and 75–25% blends. The five winter varieties are known to differ greatly in flour properties and baking quality characteristics. The three flours blended with them included (1) a low-protein good-quality soft winter composite (38535) furnished by the Mennel Milling Company, Toledo, Ohio, (2) a medium-protein very-poor-quality hard winter (38536) milled from Chiefkan, and (3) a medium-protein good-quality hard winter (39067) milled from a composite lot of grain representing approximately 7000 cars of wheat collected at the terminal markets under the supervision of the Grain Products Branch of the Food Distribution Administration, U. S. Department of Agriculture. The winter wheats

TABLE I

PROTEIN CONTENT, OPTIMUM MIXING TIME, AND BROMATE REQUIREMENT FOR EACH OF NINE FLOURS

No.	Variety	Flour protein [1]	Optimum mixing time	$KBrO_3$ requirement
		%	*min.*	*mg*
39901	Kharkof	15.7	1¾	5 to 6
39906	Cheyenne	15.3	4½	3
39907	Nebred	15.7	3¾	3
39909	Oro × Tenmarq	15.7	3½	4
39912	Chiefkan	15.2	1½	6
39722	Thatcher	14.6	3½	2 to 3
39067	Commercial composite	13.0	2½	5
38535	Soft red winter	8.2	2¼	1 to 2
38536	Chiefkan	12.2	1¼	4

[1] 13.5% moisture basis.

for these studies were grown in experimental plots in the Central and Southern Great Plains in 1939 and were milled on a Buhler mill. The flour from the spring wheat, Thatcher, generally recognized as of excellent quality for bread making, was milled from a composite lot of

[3] C.I. refers to accession number of the Division of Cereal Crops and Diseases.

four samples of grain grown in the Northern Great Plains and supplied by the Northwest Crop Improvement Association. The protein content, optimum mixing time, and bromate requirements for each of the nine flours are recorded in Table I. The laboratory numbers are also given for identification purposes later.

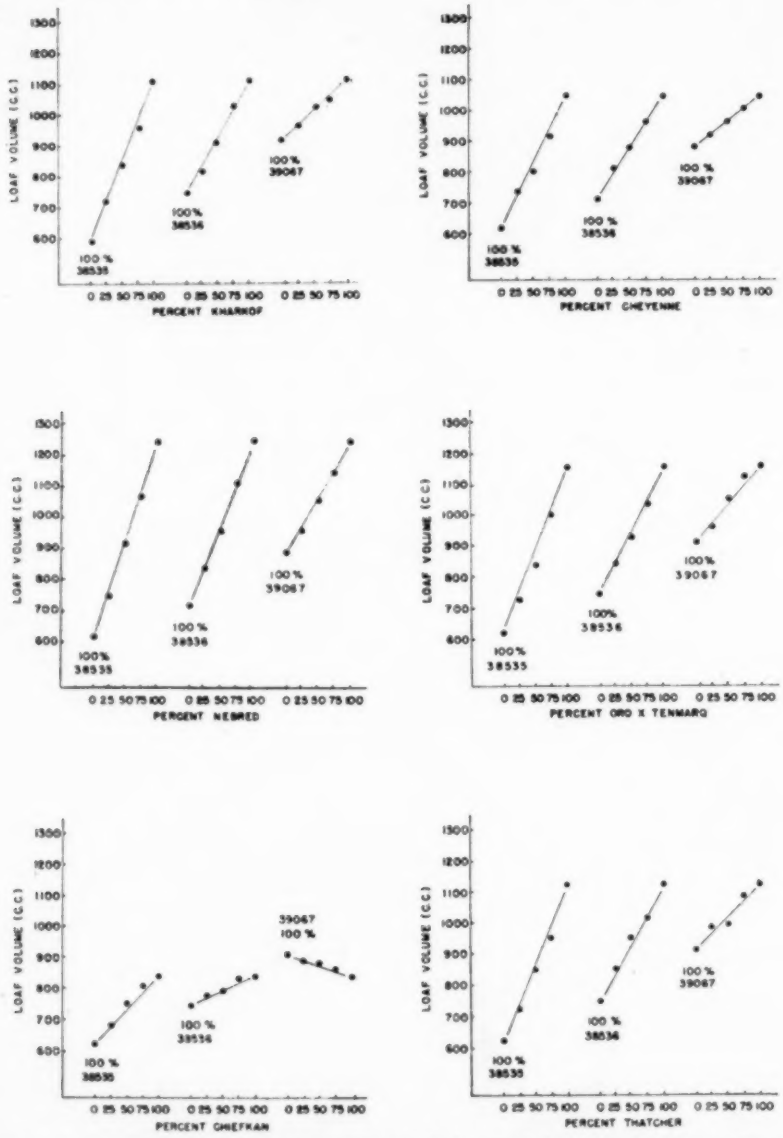

Fig. 2. Flours from one hard spring and five hard winter varieties in blends with a good-quality 8.2% protein S.R.W. (38535), a poor-quality 12.2% protein H.R.W. (38536), and a good-quality 13.0% protein commercial composite (39067).

Each original flour and blend was baked on each of three days using the A.A.C.C. straight-dough baking test procedure in conjunction with optimum mixing time (Finney and Barmore, 1941) and a baking formula (Finney and Barmore, 1939 and 1941a) containing the following ingredients per loaf: 100 g flour, water as needed, 6 g sugar, $1\frac{1}{2}$ g salt, 3 g shortening, 2 g yeast, 4 g milk solids, $\frac{1}{4}$ g 120° L malt syrup, and 3 to 6 mg $KBrO_3$, depending on the variety. The amount of $KBrO_3$ used in the blends (0 to 100%) was 3 mg for Nebred and Cheyenne, 4 mg for Oro × Tenmarq, Chiefkan, and Thatcher, and 6 mg for Kharkof. The absorptions and mixing times used for the blends were calculated, assuming each to be directly proportional to the amounts of the different flours present. These calculated properties were found to coincide with the observed experimental values, thus indicating the assumption to be correct.

Discussion

The loaf volumes for the various flour blends and for each flour when baked alone are shown in Figure 2. The percentage of each flour in the blend is given on the abscissa and loaf volume in cc on the ordinate. The lines indicate the expected loaf volumes on the basis of no complementary effects.

In Figure 3 the experimental loaf volumes of all blends are plotted against the volumes calculated on the assumption of no complementary effects. The correlation coefficient for the 54 lots was found to be + 0.988.

The data appear to provide no evidence of complementary effects. Not only is the correlation coefficient extremely high, but also there is not a single case in which the difference between actual and calculated volumes is greater than would be expected as a result of random errors.

The crumb grain scores and the crumb color scores of Oro × Tenmarq and the blends of this variety with the three blending flours are presented graphically in Figure 4. The results here shown are typical of the other varieties. It will be noted that the crumb grain scores given at the top of the figure suggest marked complementary effects, since with one exception the blends have higher scores than either flour alone. This result, however, is believed to be due to the relation between protein content and crumb grain scores. The maximum score in every case for all varieties as well as for the Oro × Tenmarq in Figure 4 was obtained for blends containing about 14% protein, which seems to represent about the optimum protein-starch ratio for the best grain scores. Blending with a lower-protein flour appears simply to reduce the protein content of the blend to a value such that the factors of high volume and high protein-starch ratio do

not function in the production of more open grains and lower scores. In this sense there is a complementary effect. It would appear, therefore, that if the tendency of high-protein flours to produce open grains and low scores is overlooked or ignored, varieties represented by high-protein flour could be erroneously evaluated, as compared with their value in blends with low-protein flour. If this relation is taken into

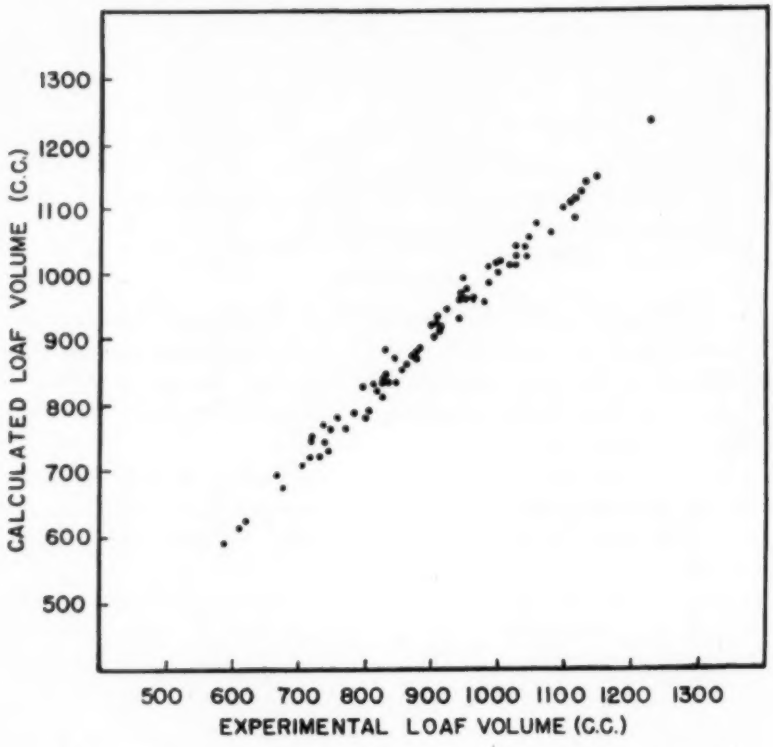

Fig. 3. Calculated and experimental loaf volumes of all flours and their blends.

account, however, there should be no confusion in the comparative evaluation of the crumb grains for different varieties.

The crumb color data for Oro × Tenmarq alone and with the three blending flours shown in the lower half of Figure 4 indicate that the crumb color of a blend is a function of the crumb colors of the flours making up the blend. Here, again, the data for Oro × Tenmarq are typical of those for the other varieties.

It seems probable that the excellent agreement between calculated and experimental loaf volumes of the blends, and the absence of complementary effects, except with respect to crumb grain, is due to the baking methods used in this laboratory. The features believed to be

important are the use of absorptions, mixing times, and bromate levels appropriate for each variety and blend, the use of milk solids to buffer any deleterious effects of overbromating, and sufficient sugar, malt,

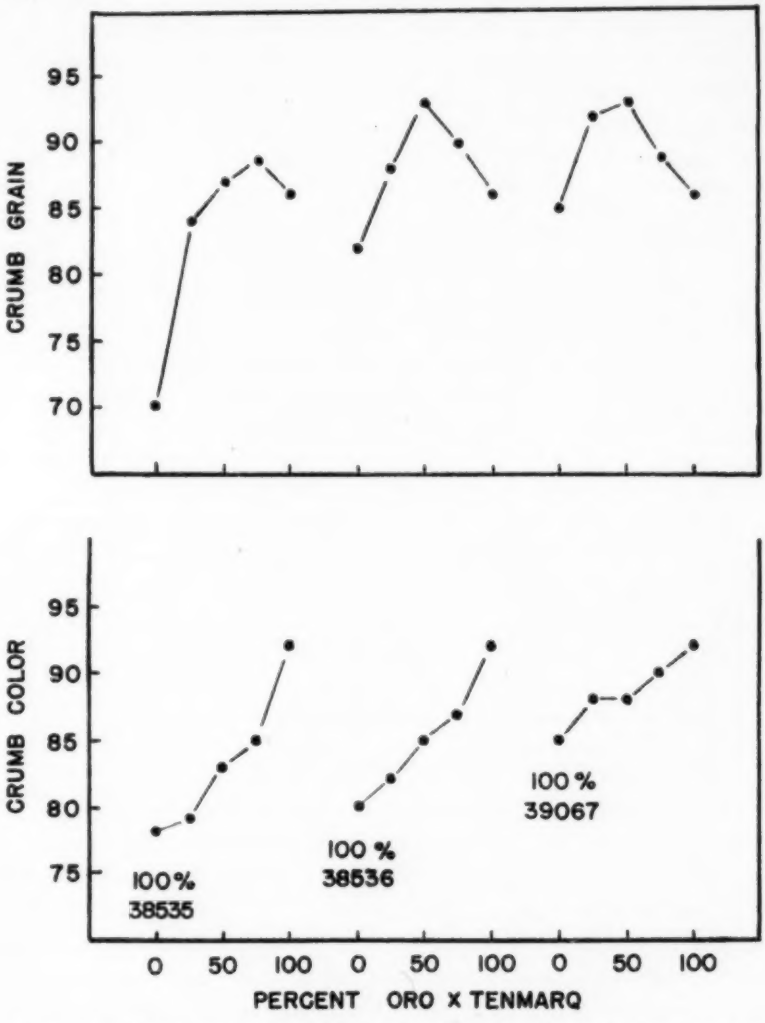

Fig. 4. Crumb grain and crumb color scores for the bread baked from Oro × Tenmarq, three blending flours, and their blends.

and yeast to insure an adequate amount and rate of gas production necessary for proper dough development. This procedure, it is believed, permits a complete or nearly complete expression of the potentialities of each variety and appears more nearly to measure "protein quality" than any other baking method used in this labora-

tory. When it is used, there appears to be left but little if any room for complementary effects on loaf volume. As noted above, the tendency of high-protein flours to produce undesirable crumb grain must be taken into account if erroneous conclusions are to be avoided. This, of course, applies to blends as well as to nonblends except that in commercial practice high-protein blends are not likely to be encountered.

Therefore, it is logical to conclude that hard wheat varieties having approximately equal protein content and equal loaf volume producing ability, in general, do not vary in their abilities to carry weaker flours. The relative strength or weakness of the various flours from the standpoint of either quality or quantity of protein is measured by loaf volume obtained by the baking test previously described. The relative ability of different flours to carry weaker flours is accurately indicated by their loaf volumes when baked alone.

TABLE II

AMOUNT OF SOFT RED WINTER WHEAT FLOUR THAT EACH OF SEVERAL VARIETIES OF HARD WHEAT FLOUR WILL CARRY AND PRODUCE A LOAF VOLUME OF 840 CC

Variety	As received			at 13% protein	
	Protein	Loaf vol.	S.R.W. carried	Loaf vol.[1]	S.R.W. carried
Nebred	15.7	1240	64	1024	45
Oro×Tenmarq	15.7	1155	60	966	37
Thatcher	14.6	1123	57	1003	43
Kharkof	15.7	1114	53	939	29
Cheyenne	15.3	1049	48	911	24
Chiefkan	15.2	840	0	763	—[2]

[1] Calculated on the assumption of linear relationships between protein content and loaf volume and a change in loaf volume for each percent protein of 80 cc for Nebred, 75 cc for Thatcher, 70 cc for Oro × Tenmarq, 65 cc for Kharkof, 60 cc for Cheyenne, and 35 cc for Chiefkan.

[2] Chiefkan alone at 13.0% protein would be expected to produce a loaf volume of only 763 cc.

Carrying Ability of Varieties. An interesting and instructive way to express the protein quality of hard wheat varieties is in terms of the percentage of a low-protein, or low-quality flour that can be blended with it and still produce a satisfactory loaf of bread. Table II shows the protein content of the flours, the loaf volumes secured in the baking tests, the loaf volumes that would be expected if each variety had the same protein content (13.0%), and the proportion of a soft red winter wheat flour that could be blended with each variety and produce a loaf with a volume of 840 cc, the latter being the loaf volume of Chiefkan, the poorest of the hard wheats. The soft red winter wheat flour is the same as that in Table I with 8.2% protein. The expected loaf volumes at 13.0% protein are calculated on the basis of the slopes of the regression lines previously determined in this laboratory for each variety.

The calculations show that Nebred can carry 64% of the 8.2%

protein soft red winter flour; Oro × Tenmarq 60%; Thatcher 57%; and finally Chiefkan, which can carry none.

On an equal protein basis of 13.0%, Nebred and Thatcher are more nearly equal and would be expected to carry 45% and 43%, respectively, of the soft red winter. Oro × Tenmarq is somewhat lower in carrying ability at 37%, Kharkof 29%, Cheyenne 24%, while Chiefkan could carry none. In fact the loaf baked from *all Chiefkan flour* containing 13.0% protein would have an expected loaf volume of only 763 cc, which is considerably less than the desired 840 cc.

Summary

Flours of one hard spring and five hard winter varieties having widely different properties, and varying in protein content from 14.6 to 15.7%, were blended in various proportions with three other flours characterized as follows: (1) a low-protein, good-quality soft red winter wheat flour; (2) a medium-protein, poor-quality hard red winter flour; (3) a medium-protein, good-quality, hard red winter flour. The individual flours and their blends were baked into bread and the results compared.

The loaf volumes and bread color scores provided no evidence of complementary effects previously reported when flours of widely different properties have been blended. Both were found to be directly proportional to the amount of each flour in the blend.

The grain scores likewise indicate no complementary effects provided allowance is made for the type of grain normally found in bread from high-protein flours. The grain in such bread is usually uneven and open, but if from flour of good quality, this characteristic disappears when blended with lower-protein flours in accordance with commercial practice, and the close, lacy, elongated cell structure characteristic of good-quality bread takes its place.

It appears that complementary effects are largely a function of the methods used in the baking test. In the work reported herein, the absorptions, mixing times, bromate levels, etc. were such that the potentialities of each variety were probably sufficiently expressed. Under such conditions there is probably less opportunity for complementary effects than would otherwise be the case.

It appears, therefore, that a flour's carrying power is a function of protein quality and quantity, and can be measured by the loaf volume obtained with the rich, highly bromated, milk-containing formula.

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THE INHIBITORY EFFECT OF A PROTAMINE FROM WHEAT FLOUR ON THE FERMENTATION OF WHEAT MASHES [1]

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Experiments already described by Stuart and Harris (1942) have demonstrated that a protamine [2] derived from wheat flour not only prevents in very small concentrations the multiplication of *S. cerevisiae*, but in greater concentrations kills this yeast. More recent experience has shown that the protein also inhibits alcoholic fermentation; *i.e.* that the yeast cells affected by it no longer ferment sugar well, if indeed at all. Although this protein exists in wheat only in combination with other substances, among them lipoids, it was a matter of some wonder that its presence did not always interfere seriously with the yields of alcohol produced by yeast fermentation. There is reason to believe that in some cases low yields of alcohol have been encountered because of this substance. But there is also abundant evidence that such is not always the case; in fact it is the exception rather than the rule.

The following study shows that inhibition of wheat fermentation by this toxic protein is a distinct likelihood, particularly when "granular flour" rather than whole wheat is used. However, some of the present methods of mashing and fermentation (perhaps accidentally) eliminate this danger. This report is therefore meant to show something of the underlying mechanism involved in the destruction of the toxic

[1] Enzyme Research Laboratory Contribution No. 84. This work done in part under Special Research Funds, SRF–2–9.

[2] The name purothionin has been proposed for this substance because of its very high content of sulfur by Balls, Hale, and Harris (1942a).

protein during ordinary fermentations, whereby its inhibitory effect is largely prevented. The experiments have been made with crystalline purothionin hydrochloride, entirely on a laboratory scale, because only laboratory quantities of the pure material were available. The principles appear to be applicable to materials in any quantity.

Purothionin has been isolated as a crystalline protamine hydrochloride from wheat flour (Balls, Hale, and Harris, 1942). It is remarkable for its high content of cystine and arginine, its power to function as an oxidation-reduction system, its basicity, and its tendency to undergo an apparent "alkaline" denaturation even at slightly acid pH levels. The toxicity of this substance appears to be due to the arrangement of the amino acids in the molecule and not to the presence of a constituent part toxic in itself. It follows that any method of breaking down the molecular arrangement destroys the toxicity. This has been previously demonstrated on small animals injected with purothionin exposed to the action of crystalline trypsin, chymotrypsin, chymopapain, and crude papain.

In the preparation and fermentation of wheat mashes two steps occur that have been found to destroy the molecular structure on which the toxicity depends: heating, and proteolysis by enzymes in the malt and possibly in the yeast. Toxicity also has been destroyed by increasing the yeast inoculum and by decreasing the acidity of the fermentation to a pH over 5. These schemes are probably just ways of increasing proteolysis, by liberating proteinase from dead yeast cells, or by producing a pH more favorable to the action of the malt enzymes. It may be concluded that the toxic protein in wheat may be removed by cooking the mash thoroughly or by the use of an actively proteolytic malt.

Procedure

Cooking: One hundred ml of water was added to 25 g of meal or flour in a 300-ml Erlenmeyer flask and cooked at various temperatures. For cooking at 80° a constant temperature bath was used and at 100° an Arnold oven was used. The cooking time was 1 hour, unless otherwise specified. Pressure cooking was done in a laboratory autoclave at 15 lb pressure per square inch.

Mashing: After cooling the cooked mash to 55°, 100 ml of malt slurry (2.5 g of ground malt soaked 1 hour at room temperature with 100 ml water) was added and the mixture kept at 55° for 1 to 1½ hours.

Fermentation: The mash was cooled to 32° and inoculated with distillers' yeast. The yeast cells were grown in wort broth for two or three days until the cell count was between 30 and 50 million cells per ml. A quantity of broth containing the desired number of cells

was centrifuged and the cells were washed into the mash with about 2 ml of water.

Alcohol Determination: The alcohol was determined as usual by distilling off about one-half of the mash, diluting the distillate to a definite volume and determining the specific gravity at 25°.

Progress of the fermentation was followed by determining the loss in weight owing to evolution of CO_2. The flasks were provided with $CaCl_2$ tubes to prevent loss of water or alcohol in the course of the fermentation. The results were not corrected for CO_2 remaining dissolved in the mash nor for that which replaced air above the surface of the liquid. They are therefore approximate.

Results

The inhibitory effect of the wheat protamine on alcoholic fermentation was found to depend on pH, the size of the yeast inoculum, and whether or not the inhibitory substance had previously been exposed to proteolytic enzymes. These effects were shown by experiments in which crystalline purothionin hydrochloride was added to sterilized (10° Bal.) malt wort. In such wort with an initial pH of 5.28 and an initial yeast count of 28×10^6 cells per ml, the concentration of purothionin critical for inhibiting fermentation (shown by gas formation within 48 hours at 32°) was between 30 μg and 40 μg per ml. With 50 μg per ml and the same yeast inoculum, gas formation was nil or slow at pH levels below about 4.7, but normal thereabove. At its normal pH of 5.28, the wort fermented vigorously when inoculated with only 2.8×10^6 yeast cells per ml, without any purothionin, but the presence of 50 μg per ml of the protamine prevented any fermentation with 10, 20, 30, or 40 times this quantity of yeast. However, vigorous fermentation occurred in the presence of the same amount of purothionin when 280×10^6 cells per ml (100 times the first inoculum) was used.

Pretreatment of wort containing 1 mg purothionin per ml (or about 30 times the critical concentration) with an equal weight of crystalline trypsin for about 3 hours at 35° (pH 5.0) permitted a vigorous fermentation after an inoculum of 26×10^6 cells per ml. The trypsin itself was not inhibitory.

Similar prevention of the inhibitory effect of purothionin was observed when it was added to miniature mashes of whole corn meal that had been malted. The concentration of inhibitor used was high (2 mg per ml of "fermenter" liquid), but it appeared to be completely destroyed by the malt proteinase.

Further experiments were then confined to miniature mashes of wheat, to which no additions of purothionin were made. These behaved as though the wheat contained the same inhibitory substance, capable of destruction by proteolysis. Thus whole wheat meal (wherein the bran contained considerable proteinase) fermented very well, even when cooked at only 80°. On the other hand, mashes of "granular flour" (an 85% extraction) fermented very poorly unless heavily inoculated with yeast or else thoroughly cooked under pressure. An experiment is shown with whole wheat in Table I and with granular flour in Table II.

TABLE I

FERMENTATION OF WHOLE WHEAT MEAL (KANSAS HARD) AFTER COOKING AT VARIOUS TEMPERATURES

Cooked for 1 hr at ° C	Loss of CO_2 after			Alcohol yield after 49 hr (ml/100 g wheat)
	6.5 hr	23 hr	49 hr	
	g	g	g	
80	0.2	5.5	6.0	31.6
80	0.1	5.6	6.1	31.6
100	0.2	5.3	5.7	30.6
100	0.1	5.6	6.1	32.6
Autoclaved	0.3	5.4	6.0	32.2
Autoclaved	0.2	5.5	5.7	31.0

Yeast inoculum was 34×10^6 cells per ml.

TABLE II

FERMENTATION OF GRANULAR FLOUR (MINN. HARD WHEAT)

Cooked for 1 hr at ° C	Yeast inoculum	Fermentation (time in hours)	Alcohol yield (ml/100 g wheat)
	Experiment I		
1. 80	26×10^6	75	2.9
2. 100	26×10^6	75	30.0
3. Autoclaved	26×10^6	75	38.3
	Experiment II [1]		
1. 80	37×10^6	42	23.0
2. 100	37×10^6	42	39.0
3. Autoclaved	37×10^6	42	37.0
	Experiment III		
1. 80	4.6×10^6	43	6.1
2. 80	9.2×10^6	43	9.2
3. 80	13.8×10^6	43	9.2
4. 80	18.4×10^6	43	14.7
5. 80	37.0×10^6	42	23.0

[1] Experiment II was a duplicate of Experiment I but a more actively growing yeast was used.

The existence of the inhibitor in wheat was most clearly demonstrated by adding dextrose and yeast, but no malt, to uncooked granular flour. The presence of the uncooked flour prevented the fermentation of the dextrose. If the flour was cooked at 80°, fermentation was poor. Cooking the flour at 100° permitted about half the dextrose to be fermented (46 hours), while autoclaving the flour allowed practically complete fermentation (Table III). However, when uncooked flour was first treated with crystalline trypsin and then mixed with yeast and dextrose under conditions comparable to the former experiment, fermentation of the sugar was rapid (Table IV).

TABLE III

FERMENTATION OF GRANULAR WHEAT FLOUR (MD. SOFT WHEAT) TO WHICH DEXTROSE [1] BUT NO MALT WAS ADDED

Cooked for 1 hr at ° C	Loss of CO_2 after		
	22 hr	28 hr	46 hr
	g	g	g
Not cooked	0.6	0.8	0.8
80	0.9	1.2	1.4
100	3.0	3.1	4.1
Autoclaved	6.4	7.7	9.2

[1] Twenty g of dextrose (about 10%) was added to each flask; the yeast concentration was 34×10^6 cells per ml mash.

TABLE IV

FERMENTATION OF DEXTROSE MIXED WITH UNMALTED, UNCOOKED GRANULAR FLOUR THAT HAD BEEN EXPOSED TO CRYSTALLINE TRYPSIN

	Loss of CO_2 after		
	1.75 hr	5 hr	44 hr
	g	g	g
1. Control	0	0	0
2. Trypsin-digest	0.7	3.6	9.1

To 25 g granular flour (soft winter wheat) 200 ml water and 200 mg wet crystalline trypsin filter cake (actually about 50 mg trypsin) were added. The pH was adjusted to 8.0. 20 g of dextrose was added and the mash kept at 37° for about 16 hours. The mash then was cooled to 32°, pH adjusted to 5.0 and inoculated with centrifuged yeast cells to make a concentration of 34×10^6 cells/ml. The control was treated in the same way except that no trypsin was added.

Summary

Evidence of a fermentation inhibitor in wheat has been presented. In the fermentation of whole wheat meal, this substance appears to be either neutralized or in some manner destroyed by other substances that must occur in the bran. In the fermentation of granular flour, the toxic substance was found to be present and active. It may be destroyed by proteolysis or by pressure cooking; partly destroyed by heating to 100° for 1 hour, but it is very little affected by a cooking temperature of 80° for 1 hour.

Acknowledgment

We wish to thank the Division of Agricultural Chemical Research, Bureau of Agricultural and Industrial Chemistry, for help in this work, particularly in the preparation of the yeast cultures.

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1942 Bactericidal and fungicidal properties of a crystalline protein isolated from unbleached wheat flour. Cereal Chem. **19**: 288–300.

BOOK REVIEW

Physical Properties of Dough. By C. O. Swanson. 258 pp. Burgess Publishing Company, Minneapolis, Minnesota. 1943. Price $2.25.

In the foreword of this book Professor Swanson states that it has been his aim to express ideas in such terms that those who have not had courses in physical and colloid chemistry may nevertheless use the book with profit. It is evident that this policy has been followed in all sections of the document, and the fundamental equations, and certain of the precise definitions of the fundamental physical properties of matter either are not included in the discussion, or are considered very briefly and in general terms.

The book opens with a discussion of certain colloidal phenomena represented in bread and dough. Then follows a consideration of starch and proteins as dough constituents. The author then returns to colloidal and related physicochemical properties, including surfaces and particles, aggregation of particles, adsorption, surface films, free and bound water, adhesion, and interfacial tension. Particular stress is laid upon the state of water in dough, and the formation and properties of dough.

The physical states of matter are mentioned in the opening paragraphs of Chapter VII, followed by a discussion of viscosity, plasticity, and elasticity. In the last two chapters these properties reappear to some degree in the author's treatment of his primary thesis, the physical tests of quality. Absorption or flour-water ratios in doughs and the use of various plastometers and penetrometers, elasticity of dough and the degree to which it is altered by various manipulations, the extensimeter, and recording dough mixers receive particular attention. Swanson refers briefly to the Hogarth, Hankoczy ("farinograph"), and Malloch recording dough mixers, but gives particular attention to the action of the Hobart-Swanson mixer, and the application of the planetary action in the mixer to the recording dough mixer which he developed. Characteristics of the graphs or "mixograms" plotted by the recording mechanism are described at some length, based upon type graphs depicted in Figure 15. The influence of protein content upon mixing behavior is stressed, and demonstrated with a collection of mixograms. Also the effects of various treatments and of certain dough ingredients upon mixing behavior are discussed.

The author agrees that the correlation between mixogram characteristics and qualities of the baked loaf has not been established abundantly. He submits further that the greatest usefulness of the mixogram lies in the information that it may furnish to supplement the data resulting from baking tests. Mixing requirements and mixing tolerance are listed among these supplementary findings. He also proposes that when a flour type is established as possessing poor baking qualities, its mixogram may be taken as a pattern of such poor qualities and it may be assumed that other flours exhibiting similar mixing characteristics are likewise inferior in baking qualities. The specifications of such mixograms are not recorded with great definiteness however.

Some omissions should be noted in Professor Swanson's treatment of physical properties of doughs. Thus there is no discussion of adhesiveness or "stickiness," or of work hardening. The latter appears to the reviewer to be of primary significance in a comprehensive survey of this subject.

Legends for certain of the figures in the book are brief and incomplete and their usefulness could be enhanced by expanding them, notably in the instance of the legends which accompany the mixograms. Also there are numerous typographical errors, but most of them are of minor consequence.

In general, Professor Swanson has provided a useful elementary discussion of the subjects included under the title of this book, and particularly for those that are not concerned with a more fundamental approach.

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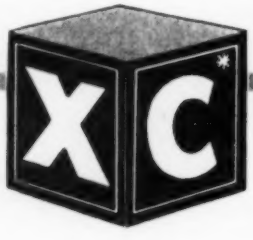

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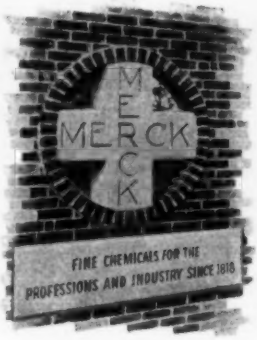

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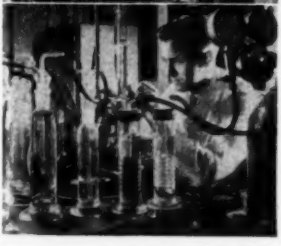

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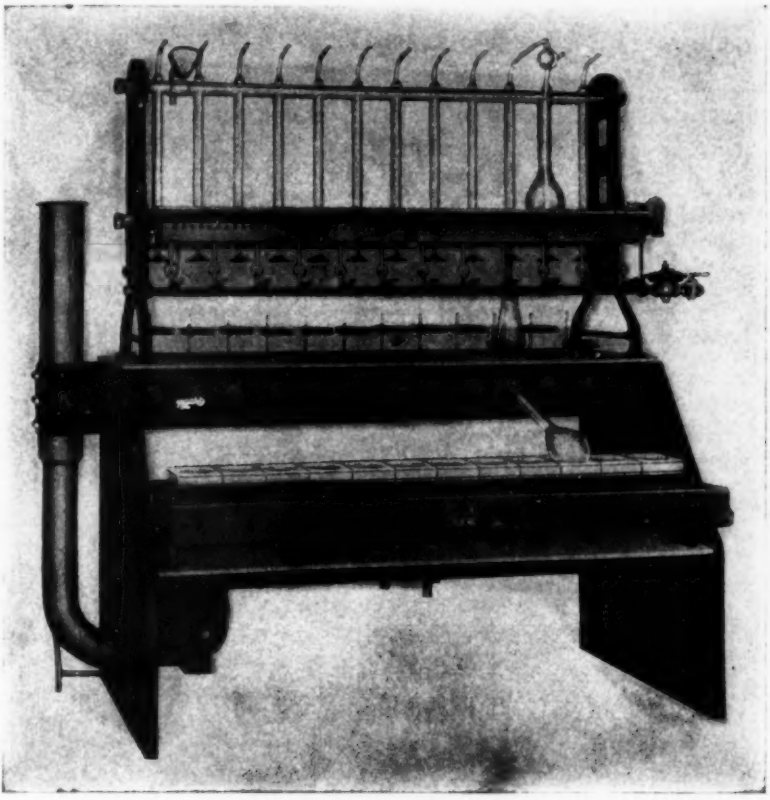

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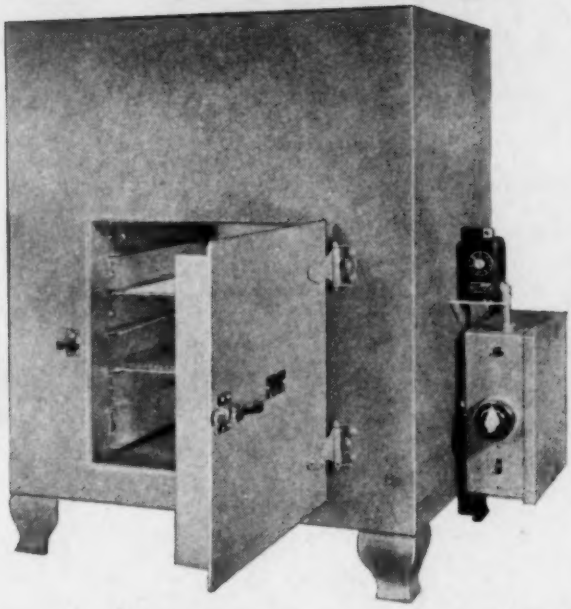

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